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Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe

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Abstract

There is a paucity of large-scale field investigations on the effects of organic toxicants on stream macroinvertebrate community structure and ecosystem functions. We investigated a total of 29 streams in two study areas of France and Finland for pesticide exposure, invertebrates and leaf-litter breakdown. To link pesticide exposure and community composition we applied the trait-based Species At Risk (SPEAR) indicator system.

In the French region, pesticide stress was associated with a decrease in the relative abundance and number of sensitive species in the communities. The presence of undisturbed upstream reaches partly compensated the effects of pesticide contamination. Functional effects of pesticides were identified by a 2.5-fold reduction of the leaf-litter breakdown rate that was closely correlated with the structural changes in the contaminated streams. No effects of pesticides were observed in Finnish streams since contamination with pesticides was very low.

In a follow-up analysis, the SPEAR approach successfully discriminated between reference and contaminated sites across different biogeographical regions, also including results of a previous field study in North Germany. Furthermore, change of the community structure was detectable at a concentration range as low as 1/100 to 1/1000 the acute 48 h-LC50 of *Daphnia magna*.

Our findings demonstrate that pesticides may influence the structure and function of lotic ecosystems and that the SPEAR approach can be used as a powerful tool in biomonitoring over large spatial scales.

Keywords: Macroinvertebrates; Aquatic; Pesticides; Recovery; Leaf-litter breakdown; Species traits

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1. Introduction

Pesticides represent a relevant stressor for many aquatic and terrestrial species (Liess et al., 2005b). They have been shown to potentially affect all groups of

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organisms in aquatic ecosystems: e.g. microorganisms (DeLorenzo et al., 2001), invertebrates (Castillo et al., 2006), plants (Frankart et al., 2003) and fish (Grande et al., 1994). Although some field studies demonstrated effects of heavy metals on the aquatic community structure at the regional scale, there is a paucity of such investigations for organic toxicants, encompassing more than one stream or river (Clements et al., 2000; Beasley and Kneale, 2003; Maret et al., 2003). Furthermore, the effects of current-use pesticides on important stream ecosystem functions such as leaf-litter breakdown (Wallace et al., 1997) are still largely unknown.

The establishment of a causal relationship between a stressor and effects can be hampered by natural variability, as every sampling site exhibits a unique combination of environmental variables and species (Liess et al., 2005b). In addition, confounding factors like the occurrence of other anthropogenic or natural stressors can mask the effects of a particular stressor. The use of species traits, such as generation time or dispersal capacity, represents an interesting approach towards encompassing both natural variability and confounding factors (Statzner et al., 2005). As most stressors or environmental gradients affect only certain trait modalities, called response traits, trait-based approaches may be used to identify the effects of a specific stressor e.g. pesticides. At the community level, the relative abundance or number of species with certain trait modalities would probably decrease thus making it possible to interpret and/or predict community change (Statzner et al., 2005). Recently, Liess and von der Ohe (2005a) developed a trait-based concept with which to distinguish pesticide effects on freshwater macroinvertebrates from the influence of other environmental variables.

This concept, called Species At Risk (SPEAR), classifies macroinvertebrates according to their vulnerability towards pesticides into sensitive species (SPEAR) and tolerant species (SPEnotAR), as evaluated by selected ecological and physiological traits. The authors successfully employed this approach in a field study on 20 streams in North Germany, where the relative abundance of SPEAR in a community declined with increasing pesticide stress. Furthermore, pesticide stress was the most important explanatory variable for different community-based SPEAR endpoints (Liess and von der Ohe, 2005a). In another study, Schriever et al. (2007) demonstrated that the highest correlation between the fraction of sensitive species and various environmental parameters was obtained for a modelled indicator of pesticide exposure, called runoff potential.

In the present study we aimed at investigating if (1) the use of the SPEAR concept in biomonitoring may be extended beyond North Germany to different biogeographical regions (Illies, 1978) and (2) pesticides have effects not only on the structure but also on the functioning of aquatic ecosystems. Therefore, we conducted field investigations in two regions of France and Finland in which the macroinvertebrate communities, leaf-litter breakdown, pesticides and physico-chemical characteristics of 29 streams were monitored during the period of pesticide application. Considering the differences in agricultural practices and especially pesticide use between these countries, we were also able to examine whether the effects of pesticides on non-target organisms are dependent on usage patterns or if the invertebrate communities adapt accordingly. To our knowledge, this is the first study that comparatively investigates pesticide effects in different biogeographical regions.

To further evaluate the performance of the SPEAR approach in large-scale biomonitoring we analyzed its ability to discriminate reference and contaminated sites across different biogeographical regions, also including the sites of the previous field study in North Germany (Liess and von der Ohe, 2005a).

2. Methods

2.1. Study area and sampling schedule

France and Finland were selected as study countries because they belong to different biogeographical regions (Illies, 1978) and exhibit contrasting pesticide use with an average of approximately 6 and 0.8 kg annually applied active ingredient per hectare, respectively (EUROSTAT, 2002). This difference partly stems from the lower prevalence of pests in Finland since the northward dispersal of many pests is averted by the colder climate. In France, Brittany in the northwest was chosen as study area because the local authorities reported frequent regional and temporal contamination of streams with pesticides from 2002 to 2004 (DIREN, 2005). A total of 16 sampling sites in first- to third-order lowland streams (Strahler, 1957) were selected which were expected to exhibit a gradient in pesticide contamination based upon the analysis of local authorities' monitoring data (Regional Agency for Agriculture and Forestry (DRAF) Bretagne, personal communication). Since Finnish agriculture is mainly localized in the southern part of the country, this region was chosen for the specification of sampling sites. 13 sites were selected in first- to third-order lowland streams covering different areas of South Finland.

All streams in the two regions of France and Finland were selected to match the physical properties of those sampled during a previous field study in North Germany

(Liess and von der Ohe, 2005a): no drying up in summer; no dredging in the present or past year; presence of adjacent fields with vegetable, corn or oil-seed crops; average stream current velocity ranging between 0.1 and 0.5 m/s; maximum stream depth of 0.8 m. Furthermore, the sites were checked in field survey and with maps (France: IGN 1:25,000 maps, Finland: Maanmittauslaitos 1:50,000 maps) to have no waste-water treatment plants, industrial facilities or mining drainage upstream. Thus, pollution other than from agricultural sources was unlikely. The location of all sampling sites is displayed in Fig. 1.

The sites were sampled before (14–19 April 2005 in France, 3–9 July 2005 in Finland) and during (19–26 May 2005 in France, 1–6 August 2005 in Finland) the estimated period of maximum pesticide contamination, according to the monitoring data from local authorities (France: DRAF Bretagne, Finland: Finnish Environment Institute (SYKE), personal communication). However, the timing of pesticide application varies and the sites may therefore have received pesticide input before the initiation of sampling. This holds especially for France, where strong rain was recorded three days before the first sampling date, possibly leading to pesticide runoff.

If stated below, we also included in the analysis the results of the study in the German region for April and May, averaged for the 3 years of study (Liess and von der Ohe, 2005a). We are aware that the results of non-randomly chosen, single regions cannot be extrapolated to the country level. However, for the ease of reading we refer to the respective region with the countries' name throughout this paper.

2.2. Physico-chemical and geographical parameters

Concentrations of oxygen, ammonium, nitrite, nitrate and orthophosphate in the stream water as well as temperature, pH and stream current velocity were measured

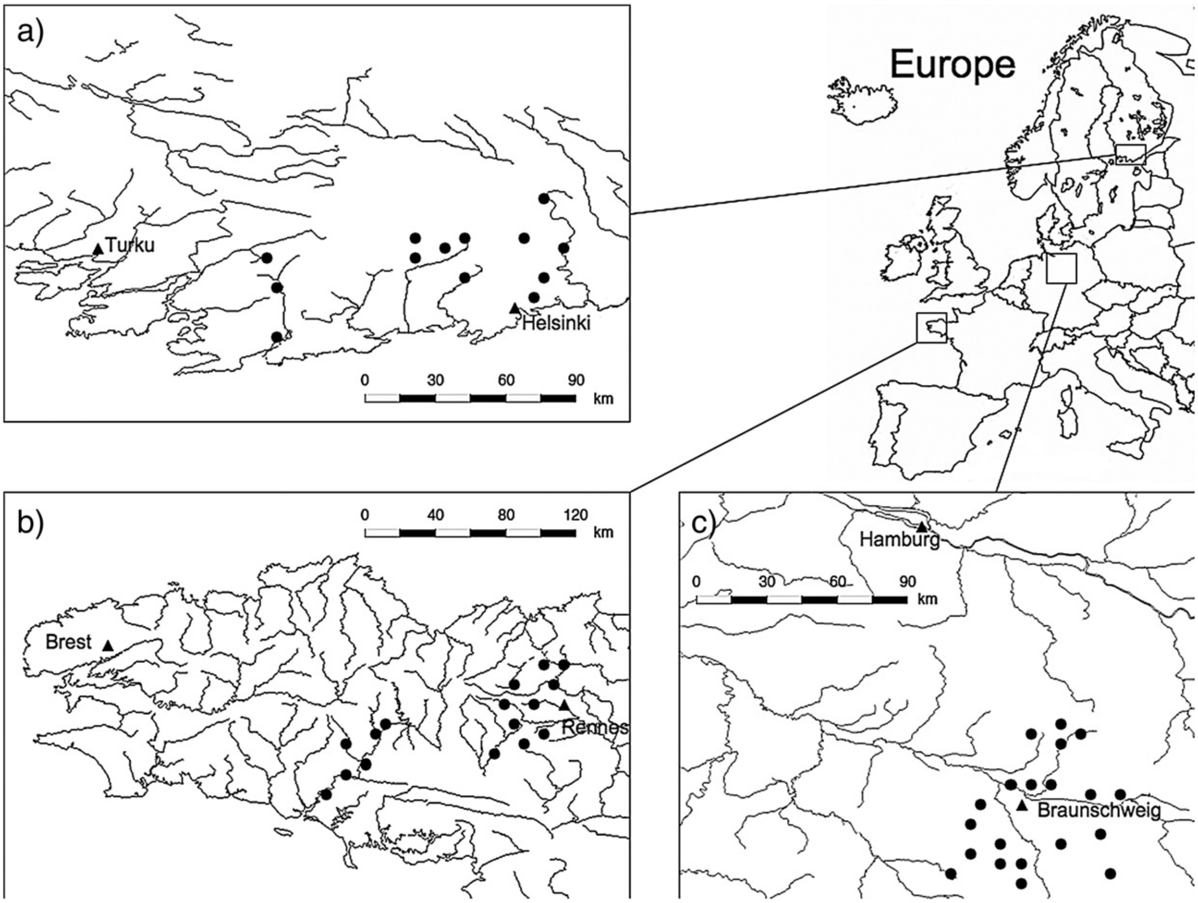

Fig. 1. Map of sampling sites and large rivers in Finland (a), France (b) and Germany (c). Sampling streams are not displayed due to scale. Regional maps were created using ESRI World Basemap Data and the European map was created with R (packages: maps and mapdata).

Table 1
Descriptive statistics of environmental parameters at the study sites in France, Finland and Germany

Parameter	Unit	France[a]					Finland[a]					Germany[b]				
		Mean	SD	% SD	Min.	Max.	Mean	SD	% SD	Min.	Max.	Mean	SD	% SD	Min.	Max.
Water temperature[c]	°C	12.58	1.21	9.65	10.55	15.40	17.74	1.89	10.64	13.30	20.10	13.30	3.00	22.56	3.50	19.50
pH[c]		6.94	0.30	4.34	6.59	7.60	6.97	0.34	4.93	6.45	7.65	7.90	0.34	4.30	6.80	8.60
Ammonium	mg/L	0.07	0.10	143.85	0.00	0.38	0.11	0.28	261.77	0.00	1.00	0.07	0.21	300.00	0	1.75
Nitrite	mg/L	0.06	0.05	78.77	0.00	0.13	0.05	0.14	273.62	0.00	0.50	0.15	0.13	86.67	0.01	0.80
Nitrate	mg/L	15.63	7.83	50.09	0.00	27.50	0.00	0.00	0.00	0.00	0.00	3.40	9.20	270.59	0.50	47.5
Orthophosphate	mg/L	1.01	0.91	90.75	0.13	3.50	0.20	0.23	114.53	0.00	0.88	0.19	0.13	68.42	0.00	0.60
Hardness[c]	°dH	6.28	2.13	33.89	3.00	9.00	4.54	0.97	21.32	3.00	6.50	Not measured				
Conductivity	µS/cm	183.23	87.72	47.87	86.00	387.00	160.54	58.96	36.72	76.00	277.00	Not measured				
Oxygen	mg/L	10.63	0.98	9.21	8.80	12.10	10.21	2.55	24.99	5.86	15.80	10.20	2.20	21.57	3.40	13.80
Current velocity	m/s	0.31	0.14	46.32	0.10	0.73	0.27	0.10	37.00	0.15	0.50	0.17	0.09	52.94	0.02	0.50
Depth	m	0.27	0.11	40.27	0.15	0.60	0.27	0.11	43.09	0.10	0.40	0.16	0.10	62.50	0.04	0.60
Width	m	2.61	1.04	39.73	1.00	4.50	2.12	0.77	36.30	1.00	3.00	1.30	.44	33.85	0.50	2.50
Tailing	%	48.28	18.11	37.52	17.50	80.00	63.08	22.78	36.11	30.00	90.00	Not measured				
Twigs	%	6.63	2.63	39.70	3.50	12.50	8.46	4.27	50.51	0.00	15.00	Not measured				
Free substrate[d]	%	64.09	15.84	24.71	30.00	78.00	72.31	14.52	20.08	45.00	85.00	Not measured				
Allochthonous leafs	%	8.28	3.38	40.83	5.00	15.00	2.69	6.96	258.40	0.00	25.00	20.00	28.00	140.00	0.00	100.00
Submersed plants[d]	%	14.75	16.36	110.88	2.50	57.50	5.00	8.16	163.30	0.00	30.00	8.00	11.00	137.50	0.00	50.00
Emersed plants	%	4.13	4.15	100.69	0.00	15.00	9.23	9.97	107.99	0.00	30.00	5.00	9.00	180.00	0.00	65.00
Filamentous algae	%	2.13	2.96	139.20	0.00	10.00	2.31	2.59	112.42	0.00	5.00	1.00	4.00	400.00	0.00	25.00
Boulder (>20 cm)	%	6.25	8.06	129.00	0.00	35.00	3.08	3.25	105.70	0.00	10.00	0.00	0.00	0.00	0.00	0.00
Cobble (5–20 cm)	%	7.50	6.83	91.08	0.00	20.00	13.46	12.81	95.16	5.00	50.00	2.00	7.00	350.00	0.00	30.00
Gravel (1–5 cm)[e]	%	15.31	10.24	66.89	5.00	30.00	13.46	8.75	65.02	0.00	30.00	5.00	10.00	200.00	0.00	40.00
Grit (0.1–1 cm)	%	21.25	9.40	44.23	10.00	50.00	16.92	10.52	62.14	5.00	30.00	Other classification				
Sand (0.01–0.1 cm)	%	27.19	13.54	49.79	10.00	55.00	28.46	13.75	48.32	10.00	60.00	24.00	37.00	154.17	0.00	100.00
Clay and silt (<0.01)[f, e]	%	22.81	15.70	68.83	0.00	50.00	24.62	18.54	75.30	5.00	65.00	55.00	46.00	83.64	0.00	100.00
Suspended matter	mL/wk	180.39	150.45	83.40	28.27	565.49	221.48	198.04	89.42	0.00	565.49	161.0	69.00	42.86	77.0	294.00
Buffer strip width[f]	m	11.56	7.85	67.88	0.00	20.00	2.60	0.97	37.31	2.00	5.00	Not measured				
Altitude	m	67.53	41.00	60.71	22.00	143.00	32.69	19.80	60.57	−5.00	68.00	Not measured				

[a] Measured twice in 2005.

[b] Measured between 1998 and 2000 and taken from the field study of Liess and von der Ohe (2005a).

[c] Intercorrelation in France (Spearman's rho > 0.8).

[d] Intercorrelation in France (Spearman's rho = −0.836).

[e] Intercorrelation in Finland (Spearman's rho = −0.802).

[f] Intercorrelation in France (Spearman's rho = −0.817).

as described in Liess and von der Ohe (2005a). Water conductivity was determined on site with a Multi 340i device of WTW (Weilheim, Germany). Total water hardness was measured in the field with an Aquamerck test (precision: 1 °dH; Merck, Darmstadt, Germany). Suspended matter was collected in suspended-matter samplers (Liess et al., 1996), measured biweekly and converted into a volume (ml) per week. In-stream structure, depth, width, tailing and buffer strip width were investigated in a 50-m reach upstream and downstream from the sampling site.

Previous studies demonstrated that the presence of forested upstream stretches that are undisturbed in terms of agricultural activities positively influenced downstream habitat quality and partly compensated for the effects of pesticides (Liess and von der Ohe, 2005a; Schriever et al., 2007). Therefore we inspected the French and Finnish streams upstream of each sampling site in field survey or with maps (France: IGN 1:25,000 maps, Finland: Maanmittauslaitos 1:50,000 maps) for the presence of riparian forests. If double-sided riparian forests at least 100 m in length were present in the 3-km reach upstream of a sampling site, we categorized it as having an undisturbed upstream reach. Modification of these criteria such as different upstream distances (2 or 4 km) or the presence of single-sided instead of double-sided forest stretches had no appreciable effects on the results of the present study. An overview of the stream characteristics, including the sites previously investigated in Germany (Liess and von der Ohe, 2005a) is given in Table 1.

2.3. Pesticide monitoring and chemical analysis

The substances for the screening programs in France and Finland were selected by (1) identifying potential compounds based upon the analysis of previous regulatory monitoring programs (France: DRAF Bretagne, Finland: Finnish Environment Institute (SYKE), personal communication) and (2) ranking them according to their toxicity, indicated by the 48-h acute median lethal concentration (LC50) for *Daphnia magna* as given in Tomlin (2001). The 10 most toxic pesticides included in the respective screening program were mainly non-polar (log $K_{ow}>4$) for Finland and polar to semi-polar (log $K_{ow}<4$) for France (Table 2). The sampling methods were arranged to catch runoff-induced exposure because this is a major input path for pesticides in small streams (Neumann et al., 2002). They were locally adapted due to differences in polarity and expected concentration levels of the compounds.

Table 2
Characteristics and measurement results of pesticides in French and Finnish streams

Compound	Monitoring program	Type [a, b]	Class [b]	LC50 (μg/L) [b]	Log K_{ow} [b]	Highest concentration (μg/L) [c]	Highest TU
Acetochlor	France	H	Chloroacetamide	9000	4.14	1.920	−3.67
Alachlore	France	H	Chloroacetamide	10,000	3.09	0.806	−4.09
Alpha-endosulfan	France	I	Organochlorine	75	4.74	0.076	−2.99
Carbofuran	France	I	Carbamate	38.6	1.52	0.715	−1.73
Chlorfenvinphos	France	I	Organic phosphorous acid	0.3	3.85	0.115	−0.42
Fenpropidine	France	F	Piperidine	500	2.59	0.059	−3.93
Linuron	France	H	Urea	120	3.00	0.097	−3.09
Oxadiazon	France	H	Oxadiazole	2400	4.91	0.071	−4.53
Pirimicarb	France	I	Carbamate	17	1.70	0.072	−2.37
Tebuconazole	France	F	Triazole	4200	3.70	0.070	−4.78
Alpha-cypermethrin	Finland	I	Pyrethroid	0.15	6.94	n.d.	n.d.
Alpha-endosulfan	Finland	I	Organochlorine	75	4.70	n.d.	n.d.
Azoxystrobin	Finland	F	Strobilurine	259	2.50	n.d.	n.d.
Cyprodinil	Finland	F	Pyrimidine	10	3.90	n.d.	n.d.
Deltamethrin	Finland	I	Pyrethroid	3.5	6.20	n.d.	n.d.
Lambda-cyhalothrin	Finland	I	Pyrethroid	0.38	7.00	n.d.	n.d.
Malathion	Finland	I	Organic thiophosphorous acid	1	2.75	n.d.	n.d.
Sulfotep	Finland	I	Organic thiophosphorous acid	2	3.99	n.d.	n.d.
Tau-fluvalinate	Finland	I	Pyrethroid	1	6.70	n.d.	n.d.
Trifluralin	Finland	F	Dinitroaniline	245	4.80	0.001 [d]	−4.34

[a] H = Herbicide, F = Fungicide and I = Insecticide.

[b] Taken from Tomlin (2001).

[c] n.d. = not detected.

[d] Time-weighted average concentration.

In France, runoff-triggered water samplers (Liess et al., 1999b) were deployed and retrieved after heavy rain events (>10 mm precipitation per day). The water samples were subsequently solid-phase-extracted using 6 ml Chromabond HR-P columns (Macherey-Nagel, Düren, Germany). Analytes trapped on the columns were extracted with 10 ml of 1:1 acetonitrile–ethylacetate and the extract gently evaporated under nitrogen to 0.3 mL. Residue analysis was conducted on an Agilent 6890N (Agilent Technologies Germany, Boeblingen, Germany) gas chromatograph (GC) linked to an Agilent 5973 mass selective detector (MSD).

In Finland, continuous water passive sampling was performed with low-density polyethylene (LDPE) strips (Booij et al., 2003), which were deployed in each stream at the beginning of the study and exposed for 28 days. LDPE strips were extracted by soaking in 500 ml *n*-hexane for 48 h. The extract was gently evaporated to 0.3 ml under nitrogen. Residue analysis was performed on an Agilent 6890N GC linked to a Pegasus III time-of-flight (TOF) mass spectrometer (Leco, Mönchengladbach, Germany). Time-weighted average (TWA) water concentrations for the LDPE samplers were calculated according to distribution coefficients from Booij et al. (2003). TWA water concentrations were converted to peak water concentrations by multiplying the TWA concentrations by a factor of 10 (Schäfer, R.B., Paschke, A. and Liess, M., unpublished data).

Although the sampling methods differed, we think that the outcomes are comparable as the results of passive sampling and runoff-triggered water sampling correlated very high (Pearson's $r=0.995$) in another study on the French streams (Schäfer et al., in preparation).

2.4. Calculation of toxicity levels

To compare the toxicity associated with the pesticide concentrations measured in the different sites, toxic units (TU) were computed from the peak water concentrations determined for each site (Liess and von der Ohe, 2005a):

$$\mathrm{TU}_{(D.\ magna)} = \max_{i=1}^{n}(\log(C_i/\mathrm{LC50}_i)) \qquad (1)$$

where $\mathrm{TU}_{(D.\ magna)}$ is the maximum toxic unit of the n pesticides detected at the considered site, C_i is the concentration (µg/L) of pesticide i and $\mathrm{LC50}_i$ is the 48 h-LC50 of pesticide i for *D. magna* (µg/L) as given in Tomlin (2001). Although peak water concentrations may have been underrated due to a delayed response of the sampling system, we assume the computed $\mathrm{TU}_{(D.\ magna)}$ to be a conservative measure of pesticide toxicity since (1) pesticide concentrations usually decrease strongly within 24 h during runoff but the 48 h-LC50 of *D. magna* was used for toxicity assessment (Richards and Baker, 1993) and (2) only the maximum toxic unit was considered instead of the sum toxicity of the pesticides detected at the respective site. If no pesticide was found a TU-value of −5 was assigned to that site, corresponding to the value found for unpolluted streams in a previous study (Liess and von der Ohe, 2005a).

2.5. Macroinvertebrate sampling

Four replicate samples (surface ca. 0.12 m^2 per sample) of different substrates were taken on each sampling date with a 500-µm mesh-size Surber Sampler (Hydro-Bios, Kiel, Germany) and preserved with formalin (ca. 4% vol.). The invertebrates were sorted out, counted and identified to the lowest possible taxonomic level, which was genus for most taxa. A list of the taxa found in France and Finland along with their frequency in the samples is given in the Supplementary material.

2.6. SPEAR-index calculation and endpoints

The identified taxa were classified into SPEAR and SPEnotAR according to ecological and physiological traits as described in Liess and von der Ohe (2005a). Since life-cycle traits such as emergence time and voltinism are dependent on the biogeographical region (e.g. Central and Northern Europe), the classification for a particular taxon may differ between regions. The available data and region-dependent classification information are compiled in a database which is publicly available and comprises about 1000 macroinvertebrate taxa (Liess et al., 2006). After classification into SPEAR and SPEnotAR the relative abundance of taxa which are potentially sensitive towards pesticides in a community, was computed for each site and date:

$$\%\mathrm{SPEAR}_{(\mathrm{abundance})} = \frac{\sum_{i=1}^{n} \log(x_i + 1)\cdot y}{\sum_{i=1}^{n} \log(x_i + 1)}\cdot 100 \qquad (2)$$

where n is the number of taxa, x_i is the abundance of taxon i and y is: 1 if taxon i is classified as SPEAR, otherwise 0. Similarly, we calculated the values for another community endpoint $\%\mathrm{SPEAR}_{(PM\ \mathrm{abundance})}$, where classification of taxa relies only on physiological sensitivity (P) and migration ability (M) to exclude biogeographical bias of this index. $\%\mathrm{SPEAR}_{(PM\ \mathrm{abundance})}$ was used to examine the applicability of the SPEAR concept across different regions. The endpoint $\%\mathrm{SPEAR}_{(\mathrm{number})}$, which indicates the relative number of sensitive taxa, was computed for each site and date by:

$$\%\mathrm{SPEAR}_{(\mathrm{number})} = \frac{\sum_{i=1}^{n} y}{n} \cdot 100. \tag{3}$$

Finally, the endpoint $\mathrm{SPEAR}_{(\mathrm{abundance\ during/before})}$ was computed by dividing the $\%\mathrm{SPEAR}_{(\mathrm{abundance})}$ during the period of maximum pesticide input in streams (France: May, Finland: August) by $\%\mathrm{SPEAR}_{(\mathrm{abundance})}$ before this period (France: April, Finland: July). Time periods were estimated on the basis of information from local authorities and as reported elsewhere (Liess et al., 1999b; Liess and von der Ohe, 2005a).

2.7. Leaf-litter breakdown

Three grams of air-dried *Alnus glutinosa* leaves at abscission was anchored to the streams in coarse (mesh size: app. 6 mm; polyethylene bag size: 20×20 cm) and fine (mesh size: 50 μm; nylon cylinder size: 15 cm length, 7.5 cm diameter) enclosures. Leaves in the coarse bags were accessible to invertebrates whereas leaves in the fine bags were not and served as control for microbial degradation and leaching (Gessner and Chauvet, 2002). Triplicate coarse and fine bags were deployed after the first sampling for approximately 21 days in 12 randomly selected sites in France and in 8 sites in Finland. To correct for handling losses three coarse and fine bags were treated the same way as the others but returned immediately to the laboratory after a brief deployment in the stream. After retrieval of the bags, the remaining litter was washed, oven-dried to a constant mass at 60 °C (24 to 48 h), reweighed and averaged for each type of bags for every station. The remaining leaf mass $W_t(s)$ in grams for station s after time t was obtained by summing up the handling-loss corrected weight of the remaining litter at site s from the coarse enclosures and the loss due to microbial degradation and leaching at site s derived from the fine enclosures (for details see Benfield (1996)). The exponential leaf breakdown rate k_s was computed by:

$$k_s = \frac{-\ln\left(\frac{W_t(s)}{W_0(s)}\right)}{t_s} \tag{4}$$

where $W_0(s)$ is the initial leaf mass in grams at site s and t_s is the deployment time for the considered site (other variables as defined above) (Benfield, 1996).

2.8. Data analysis

The data analysis was performed separately for the Finnish and French sites if not otherwise indicated. Prior to analysis, the average values for the two sampling dates were calculated for all variables that were measured twice at each site, in order to avoid temporal pseudoreplication. However, we also conducted the analyses for each single measurement date. The results broadly confirmed the findings of the analyses performed for the combined data set and they are therefore not shown. Hierarchical cluster analysis of environmental variables using Spearman's rho as similarity measure was performed to check for collinearity and redundancy among environmental variables (McGarigal et al., 2000). Environmental variables that exhibited strong correlation with other variables (Spearman's rho > 0.8; see Table 1) but were implausible an explanation of differences in macroinvertebrate assemblages, on the basis of common ecological knowledge (Allan, 1995), were removed from the data set to avoid misspecification of the linear model (Flack and Chang, 1987; McGarigal et al., 2000).

Multiple linear regression was applied to identify the environmental variables that were best suited to explain the different SPEAR metrics and the leaf decomposition rate k. We weighted the sites in the regression according to the total log $(x+1)$ abundance of species (only for SPEAR metrics). We employed manual model building, defining models on the basis of expert judgement and automatic model building starting with the null model (no explanatory variable included) or the full model (all explanatory variables included). The statistical procedure was backward and forward entering of variables with Akaike's Information Criterion as stepwise model selection criterion (Akaike, 1974). Model simplification was performed using t-test for significance of single variables and analysis of variance (ANOVA) with F-test for significance of the complete model. Models with different numbers of parameters were compared with the F-test. Goodness of fit was assessed with the adjusted r^2 (r^2 for models with only one explanatory variable). Analysis of covariance (ANCOVA) with t-test was applied to check for significant differences in slope or intercept for factors in regression. Model checking included: heteroscedasticity, normal distribution of residuals and influence of single observations using residual-leverage plots and Cook's distance. We applied hierarchical partitioning to determine the relative importance of independent explanatory variables in the linear models (Chevan and Sutherland, 1991).

To detect effects of pesticide input on SPEAR metrics or k in a single country, the respective values were split according to the $\mathrm{TU}_{(D.\ magna)}$ into sites potentially receiving ($\mathrm{TU}_{(D.\ magna)} > -3.5$) and potentially not receiving ($\mathrm{TU}_{(D.\ magna)} < -3.5$) pesticide input. Welch's t-test for unequal variances was used to compare the means of the two groups.

For the comparison of SPEAR metrics across countries, the respective observations of France, Finland and Germany were grouped according to their $\mathrm{TU}_{(D.\ magna)}$ as:

- reference ($\mathrm{TU}_{(D.\ magna)} < -3.5$)
- lightly contaminated ($-3.5 < \mathrm{TU}_{(D.\ magna)} < -2.25$)
- and heavily contaminated sites ($\mathrm{TU}_{(D.\ magna)} > -2.25$).

The class boundaries (−3.5 and −2.25) were chosen to make the sample sizes as even as possible for all countries. However, the use of different class boundaries (−4 and −2) yielded the same results. To detect significant differences between means of groups, non-parametric ANOVA with Kruskal–Wallis test was conducted, followed by a non-parametric multiple comparison test of the Behrens–Fischer type (Munzel Hothorn, 2001).

All statistical computations and graphics were created with the open source software package R (R Development Core Team, 2006) using version 2.4.0 (for Mac OS X, 10.4.8) with appropriate additional packages (hier.part, Hmisc, npmc, maps and mapdata).

3. Results

3.1. Characterization of investigated streams and communities

Water temperature, pH, hardness and oxygen exhibited only slight variation (up to 34% standard deviation) among the sampling sites in each study area, while streambed substrate composition showed the largest variability (up to 400% standard deviation) (Table 1). The French and German streams were very similar concerning most stream characteristics but differed in the clay and silt content of the substrate. In contrast, the French and Finnish sites were very similar regarding substrate composition but mainly differed in water chemistry characteristics. A total of 94 different taxa with an average of 27 taxa per stream were identified in the 13 Finnish streams; the values for the 16 French streams were 110 and 33, respectively (see Supplementary material). In the study of Liess and von der Ohe (2005a) 129 different taxa and an average of 24 taxa per sampling site were found in the German streams, applying the same level of taxonomic identification as in the present study.

In Finland, *Asellus aquaticus*, Chironomidae spp., *Dryopoidea* spp., *Leuctra fusca*, Limoniidae spp., Oligochaeta spp. and Simuliidae spp. were found in more than 85% of the samples. The most common taxa (>85% of samples) in France were *Baetis rhodani*, Elmidae spp., *Ephemerella ignita*, *Erpobdella* spp., *Gammarus pulex*, *Hydropsyche* spp. and Oligochaeta spp.. In the German study, Chironomidae spp., *Erpobdella octoculata*, *Glossiphonia complanata*, Tubificidae spp., *Gammarus pulex* and *Limnephilus lunatus* were present in more than 85% of the samples.

3.2. Pesticide monitoring

In Finland, only the fungicide trifluraline was detected; it had a maximum TWA water concentration of 1.11 ng/L (Table 2), but resulted in a neglectable $\mathrm{TU}_{(D.\ magna)}$ for the Finnish streams (Fig. 2). In contrast, all pesticides of the monitoring program were detected in samples from French streams (Table 2). They were identified and quantified after the only strong rain event (20 to 30 mm rainfall between May 12 and 13, 2005 (Meteo France, 2006)) that occurred in the study area during the sampling period. For the French sampling sites pesticide concentrations with $\mathrm{TU}_{(D.\ magna)}$ values up to −0.42 were observed (Fig. 2). For the German streams $\mathrm{TU}_{(D.\ magna)}$ values up to −0.71 were reported (Liess and von der Ohe, 2005a).

3.3. Relationship between environmental variables and SPEAR metrics

For the Finnish streams, no significant linear model could be established for $\%\mathrm{SPEAR}_{(\mathrm{abundance})}$, and stream depth was the only variable to explain $\%\mathrm{SPEAR}_{(\mathrm{number})}$ (Table 3). Neither $\%\mathrm{SPEAR}_{(\mathrm{abundance})}$ nor $\%\mathrm{SPEAR}_{(\mathrm{number})}$ was significantly different for streams with and without

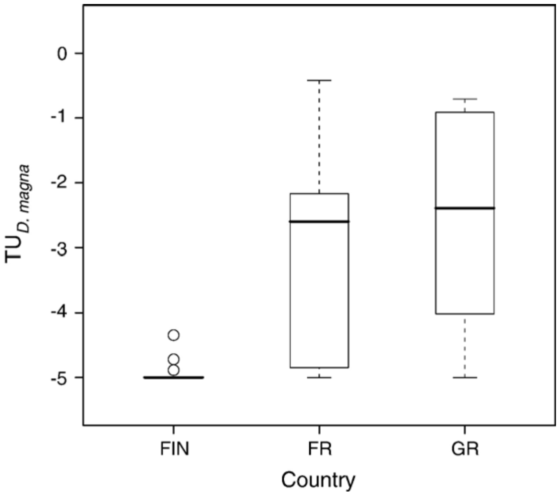

Fig. 2. Box–Whisker plot of Toxic $\mathrm{Unit}_{(Daphnia\ magna)}$ for the sites in the study areas of Finland (FIN), France (FR) and Germany (GR).

Table 3

Summary statistics of linear models to explain SPEAR endpoints and leaf-litter breakdown rate *k* in French and Finnish streams

Response variable	Country	Model statistics			Relative importance of explanatory variable in best-fit linear model (%)[a]							
		Adj. r^2	*n*	*P*	Toxic unit	Undisturbed upstream reach	Stream depth	% of filamentous algae	Water temperature	Current velocity	$\%SPEAR_{(abundance)}$	% of sand
$\%SPEAR_{(abundance)}$	Finland	–	13	>0.05	–	–	–	–	–	–	Not explanatory variable	–
$\%SPEAR_{(number)}$	Finland	0.56[b]	13	0.003	–	–	100	–	–	–		–
$\%SPEAR_{(abundance)}$	France	0.64	16	<0.001	61.80	31.20	–	–	–	–		–
$\%SPEAR_{(number)}$	France	0.98	16	<0.001	14.30	29.90	–	19.70	21.40	14.70		–
k	Finland	0.88[b]	8	<0.001	–	–	–	–	100	–	–	–
k	France	0.44[c]	12	0.041	51.75	48.25	–	–	–	–	–	–
k	France	0.94	12	<0.001	–	–	–	–	–	–	79.8	20.2

[a] Determined in hierarchical partitioning (Chevan and Sutherland, 1991).

[b] r^2 not adjusted for one explanatory variable.

[c] Not best-fit model.

undisturbed upstream reaches (Welch's *t*-test, $P=0.439$ and $P=0.696$).

For the French streams, variability in $\%SPEAR_{(abundance)}$ was best explained by $TU_{(D.\ magna)}$ and the factor undisturbed upstream reach (Table 3). The negative relationship between $\%SPEAR_{(abundance)}$ and $TU_{(D.\ magna)}$ is illustrated in Fig. 3.

Values for $\%SPEAR_{(abundance)}$ and $\%SPEAR_{(number)}$ were significantly reduced for streams which received pesticide input (Welch's *t*-test, both $P<0.001$). Pesticide-impacted streams with undisturbed upstream reaches had significantly higher $\%SPEAR_{(abundance)}$ and $\%SPEAR_{(number)}$ compared to impacted streams which lacked these reaches (Welch's *t*-test, $P=0.017$ and $P<0.001$).

3.4. Relationship between leaf-litter decomposition and environmental variables

The leaf-litter breakdown coefficient *k* for the Finnish streams ranged from 0.001 to 0.046 and *k* was highly correlated with water temperature (Table 3). For the French streams, *k* values between 0.008 and 0.067 were measured. The breakdown rate was significantly different between streams potentially receiving (0.0252±0.0049 s.e.) and not receiving pesticide input (0.0588±0.0008 s.e.) (Welch's *t*-test, $P<0.001$). $TU_{(D.\ magna)}$ and the factor undisturbed

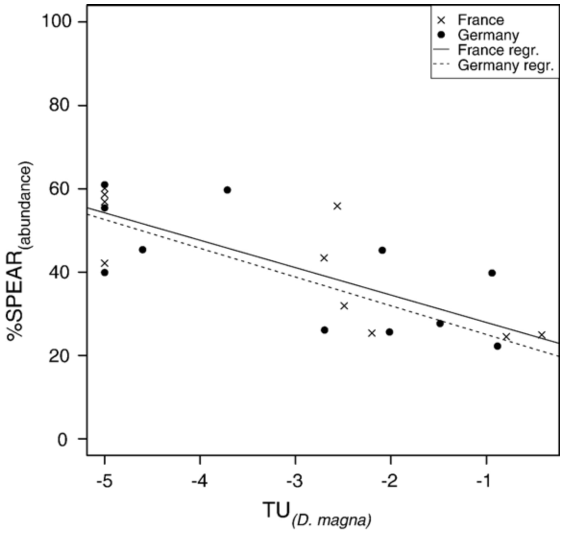

Fig. 3. Relation between the benthic invertebrate community structure expressed as $\%SPEAR_{(abundance)}$ and the Toxic Unit$_{(Daphnia\ magna)}$ of the sites with undisturbed upstream reaches in France and Germany. Linear regression lines are significant with $P<0.001$, $r^2=0.61$ and 0.64 for French ($n=10$) and German streams ($n=11$), respectively. The slopes and intercept are not significantly different (analysis of covariance, $P=0.581$).

upstream reach could explain a significant part of the variation in k (Table 3), where k responded positively to the presence of undisturbed upstream reaches and negatively to an increase of pesticide stress. However, the best-fit model for k only comprised the variables %SPEAR$_{(\text{abundance})}$ and % of sand on the stream bottom (Table 3). This indicated that pesticide stress had no direct effect on the leaf-litter breakdown but mediated through its negative effect on sensitive species. The relationship between %SPEAR$_{(\text{abundance})}$ and k is displayed in Fig. 4.

3.5. Comparison of %SPEAR$_{(PM\ \text{abundance})}$ across geographical areas

For Germany, France and Finland, the means of %SPEAR$_{(PM\ \text{abundance})}$, a metric excluding traits with biogeographical variability, were significantly different when grouped by TU$_{(D.\ magna)}$ (Kruskal–Wallis test, $\chi^2_{6,49}=26.32$, $P<0.001$). All the reference sites in the three countries (TU<-3.5) exhibited about the same mean level of %SPEAR$_{(\text{PM abundance})}$ with 46, 52 and 54% for France, Finland and Germany, respectively. Pairwise comparisons showed that values from reference sites were significantly different ($P<0.05$) from the values recorded in the highly contaminated sites in France and Germany (TU>-2.25) (Fig. 5). For all countries, neither the reference sites nor the highly contaminated sites were significantly different ($P<0.05$) from the mean %SPEAR$_{(PM\ \text{abundance})}$ of lightly polluted streams ($-3.5<\text{TU}<-2.25$). Nevertheless, a clear decline of the %SPEAR$_{(PM\ \text{abundance})}$ was visible for the group of lightly polluted sites compared to reference sites. Furthermore, when the data from all countries were pooled, the difference between lightly polluted and reference sites showed to be significant in multiple comparisons ($P=0.003$).

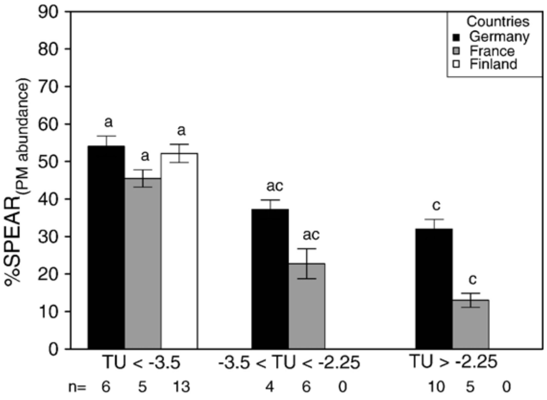

Fig. 5. Relation between %SPEAR$_{(PM\ \text{abundance})}$ and Toxic Unit$_{(Daphnia\ magna)}$ (TU) for the study sites in France, Finland and Germany with the sample sizes n. Different letters over the bars indicate significant differences in multiple comparison post-hoc test (non-parametric Behrens–Fischer test, $P<0.05$). Error bars show standard error.

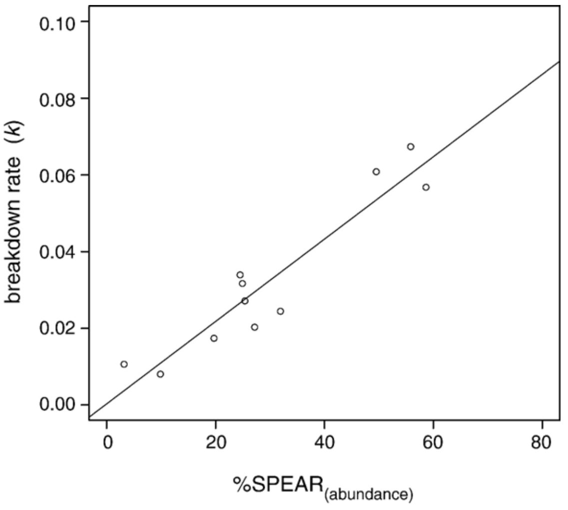

Fig. 4. Relation between leaf-litter decomposition and %SPEAR$_{(\text{abundance})}$ for 11 streams in Brittany, France. Linear regression was significant with $P<0.001$ and $r^2=0.89$.

%SPEAR$_{(PM\ \text{abundance})}$ was lower for France than for Germany concerning the groups of lightly and highly contaminated streams (Fig. 5), because of the higher TU$_{(D.\ magna)}$ values of the French sites in each group. No difference was observed in regression analysis (not shown, but compare Fig. 3).

3.6. Temporal change of %SPEAR$_{(abundance)}$

For Finland, the average change of the communities from July to August, SPEAR$_{(\text{abundance during/before})}$, for all streams was 1±0.06 standard error (s.e.). Hence, no change in the community structure occurred in the period of pesticide application. Similarly, French streams that were not subject to pesticide input (TU$_{(D.\ magna)}<-3.5$) had an average SPEAR$_{(\text{abundance during/before})}$ of 1.01±0.08 s.e., while stations which received pesticide inputs (TU$_{(D.\ magna)}>-3.5$) had a mean SPEAR$_{(\text{abundance during/before})}$ of 0.92±0.06 s.e.. However, SPEAR$_{(\text{abundance during/before})}$ values for the two groups were not significantly different (Welch's t-test, $P=0.215$).

The average values of SPEAR$_{(\text{abundance during/before})}$ for the German streams were 0.92±0.06 s.e. and 0.74±0.08 s.e. for streams potentially not receiving and receiving pesticide input, respectively. These values were significantly different at $P=0.048$ (Welch's t-test).

The pooled data for community change in the period of pesticide application (SPEAR$_{(\text{abundance during/before})}$) of all regions were significantly different concerning the grouping by $TU_{(D.\ magna)}$ (Kruskal–Wallis test, $\chi^2_{2,49}=6.94$, $P<0.031$). In multiple comparison tests, the medium ($P=0.032$) and highly contaminated sites ($P=0.025$) differed significantly from the reference sites, indicating an acute response of $\%SPEAR_{(abundance)}$ from pre- to pesticide-application period.

4. Discussion

4.1. Linking pesticide input to community composition

In the present study we conducted field investigations in a region of each France and Finland to examine whether current-use pesticides would affect freshwater macroinvertebrate communities. Pesticide stress in terms of $TU_{(D.\ magna)}$ was almost absent in the Finnish streams, and the variation in the lotic macroinvertebrate community could not be attributed to the presence of these contaminants. The results of our pesticide measurements are in agreement with those of a Finnish governmental screening program from 2004 to 2005 (Finnish Environment Institute (SYKE), personal communication), and we do not know of any field study on Finnish streams reporting pesticide concentrations at a level potentially toxic to invertebrates. The low pesticide input in Finnish streams compared to the French may be attributed to the reduced pesticide-application rate, different compound classes and geological factors, e.g. higher organic carbon content of the Finnish soils (European Commission Joint Research Centre, Institute for Environment and Sustainability, personal communication). We suppose that the results presented here are representative for the general situation in the agricultural region of Finland, since we sampled streams in different areas of this region. Nevertheless, a more thorough investigation including more compounds, sampling sites and a longer sampling period could alter this appraisal.

For the French streams, we found a clear relationship between pesticide stress and community composition as indicated by $\%SPEAR_{(abundance)}$. Among the environmental variables, pesticide stress, indicated by $TU_{(D.\ magna)}$, best explained the results for $\%SPEAR_{(abundance)}$. Furthermore, we observed a decline in the relative abundance of sensitive taxa after pesticide runoff, although it was not significant. This may be due to effects on the communities before the beginning of our study.

The concentrations presumed to cause an effect in the French streams, resulted in $TU_{(D.\ magna)}$-values up to -0.42 (Table 2) and are in accordance with those reported elsewhere. Liess and Schulz (1999a) showed that pesticide-contaminated runoff water with $TU_{(D.\ magna)}$ up to 0.38 was the main cause for the decline in abundances of several macroinvertebrate species in a small headwater stream. The study of Liess and von der Ohe (2005a) demonstrated strong causality between a $TU_{(D.\ magna)}$ larger than -3 and a decline of SPEAR. Castillo et al. (2006) stated change in the invertebrate community structure associated with an exposure to pesticide concentrations in the surface water up to a $TU_{(D.\ magna)}$ of -0.73. Finally, a review of mesocosm studies reported effects on the macroinvertebrate community above a $TU_{(D.\ magna)}$ of -2 (van Wijngaarden et al., 2005).

The relationship between the relative abundance of sensitive taxa and pesticide stress was similar for the French and German streams (Fig. 3). Concurrently, geographical and physico-chemical variables varied and approximately 35% of the taxa found in France were not recorded in the German streams. Hence, the response of traits to pesticide stress was not affected by geographical and taxonomical differences.

The insensitivity of traits to a range of environmental gradients was also reported by Charvet et al. (2000). This issue warrants further investigation, especially in South and non-European regions, because if the dose–response relationship between traits and pesticide stress could be extrapolated to a wider geographical scale, it would constitute a powerful tool for an effect assessment on the continental scale.

4.2. Effects of pesticides on leaf-litter decomposition

In our study on the French streams, we found a significant decrease in leaf-litter decomposition rates for leaves of *A. glutinosa* due to pesticide stress. The decomposition rates for streams potentially not receiving pesticide input (0.0588 ± 0.0008 s.e.) are in the range of values reported for relatively pristine streams in Portugal during summer (0.051 to 0.064) (Graca et al., 2001). Comparison of the ratio of breakdown coefficients at sites potentially receiving and sites potentially not receiving pesticide input (0.42) with the threshold values proposed by Gessner and Chauvet (2002) confirms the functional impairment caused by pesticides (ratio <0.5 indicates severe impairment). Chung et al. (1993) also reported a ratio of 0.35 of breakdown rates of rhododendron leaves for a pesticide-treated stream and a reference stream.

A reduction in $\%SPEAR_{(abundance)}$ and $\%SPEAR_{(number)}$ (not shown) was closely related to a decrease in leaf-litter processing rates (Fig. 4). This may be explained by the fact that 2/3 of the shredder taxa (classified according to Merritt and Cummins (1996)) belong to SPEAR. Maltby et al. (2002) also reported high positive correlation (Pearson's $r=0.83$) between physiological effects of pesticides on a shredder species and impairment of leaf-litter processing. To sum up, pesticide input in the streams of Brittany, France also affected an important ecosystem process, leaf breakdown, probably mediated through the adverse effects on number and abundance of SPEAR taxa.

4.3. Undisturbed upstream reaches enhance quality of impacted streams

The presence or absence of riparian forest parts in the 3-km upstream reach explained a significant part of the variation in the SPEAR endpoints for the French sampling sites. The presence of undisturbed upstream reaches lead to significantly higher values of $\%\mathrm{SPEAR}_{(\mathrm{abundance})}$ and $\%\mathrm{SPEAR}_{(\mathrm{number})}$ at contaminated sites. Other authors also reported the relevance of undisturbed upstream reaches for recovery from disturbance.

A study on the Suna river (Japan) attributed the recovery of several invertebrate species after pesticide spraying to recolonization from unsprayed upstream reaches within a 5-km distance (Hatakeyama and Yokoyama, 1997). Liess and von der Ohe (2005a) demonstrated the recovery of pesticide-impacted communities when riparian forest reaches were available in the 4 km upstream reach. A recent study, linking exposure modelling with macroinvertebrate composition of 360 streams investigated over a 17-year period in North Germany, showed that the presence of forest parts in the 1.5 km upstream reach facilitated recovery of the relative abundance of SPEAR taxa after modelled pesticide contamination (Schriever et al., 2007).

Two mechanisms could explain this positive impact of undisturbed upstream reaches. First, undisturbed upstream reaches may provide recolonization pools from which species could drift downstream to the impacted reach (Waters, 1972). Second, input of woody debris and leaf litter from the riparian forest might supply more energy for the downstream reaches and thus increase number and abundance of taxa (Wallace et al., 1995; Bond et al., 2006). The latter mechanism should also increase the number and abundance of sensitive species at slightly or non-contaminated sites. However, Schriever et al. (2007) reported no significant difference in $\%\mathrm{SPEAR}_{(\mathrm{abundance})}$ for low-contamination streams with and without undisturbed upstream reaches in the period before pesticide application. In the present study also, we did not find significant differences in abundance or number of SPEAR taxa for uncontaminated sites having and not having undisturbed upstream reaches in Finland. Therefore, current evidence indicates that recovery should mainly be attributed to instream recolonization by macroinvertebrates from undisturbed upstream reaches, although a more thorough study is still necessary to clarify this issue. However, regardless of the underlying mechanism, undisturbed upstream reaches clearly enhance recovery of impacted reaches and this poses a valuable management tool for freshwater conservation in agricultural areas.

4.4. Derivation of an effect threshold for pesticides

For the pooled data of the German, French and Finnish streams, a significant reduction of sensitive taxa was observed for $\mathrm{TU}_{(D.\ magna)}$ values higher than −3.5. However, this value is only a rough estimate for a threshold value because there were hardly any observations for $\mathrm{TU}_{(D.\ magna)}$ in the interval (−3, −5). Furthermore, regarding the dose–response relationship between $\mathrm{TU}_{(D.\ magna)}$ and $\%\mathrm{SPEAR}_{(\mathrm{abundance})}$ in France and Germany (Fig. 3) it remains open, if an effect threshold exists or if the relationship is continuously linear up to a $\mathrm{TU}_{(D.\ magna)}$ of −5. Thus, more field data are needed especially for low values of $\mathrm{TU}_{(D.\ magna)}$ to clarify this issue. Nevertheless, our data indicate effects of pesticides in the $\mathrm{TU}_{(D.\ magna)}$ range of −2 and −3. Two other field studies also reported shifts in the invertebrate assemblages for $\mathrm{TU}_{(D.\ magna)}$ between −2 and −3 (Berenzen et al., 2005; Liess and von der Ohe, 2005a). In contrast, we do not know of any mesocosm study where effects below a $\mathrm{TU}_{(D.\ magna)}$ of −2 were found (van Wijngaarden et al., 2005). However, most studies on stream mesocosms just deal with a single pulse exposure while in the field repeated input of many different pesticides frequently occurs (van Wijngaarden et al., 2005). Recently, a laboratory study with *D. magna* showed that a repeated exposure to dimethoate and pirimicarb significantly increased mortality (Andersen et al., 2006). In addition, multiple stressors may occur in areas with intense agriculture (e.g. pesticides with a different mode of action, chronic ammonium exposure, eutrophication) and act additively or synergistically, which is commonly not incorporated in mesocosm studies (Heugens et al., 2001). For example, organophosphorous pesticides that were also detected in the French streams (Table 2) have been demonstrated to elicit greater-than-additive responses in combination with various herbicides (Lydy and Austin, 2005).

We suggest that the community change at $\mathrm{TU}_{(D.\ magna)}$ values <-2 may have resulted from the long-term propagation of sublethal effects. This hypothesis is supported by the fact that the relative abundance of sensitive taxa exhibited only a small acute response to pesticide stress in lightly contaminated streams (17 ± 10.1% reduction in $\%\mathrm{SPEAR}_{(\mathrm{abundance})}$). Sublethal effects like reduced fecundity or delayed emergence are known to appear up to a $\mathrm{TU}_{(D.\ magna)}$ of −4 (Liess, 2002) and if they cause a competitive disadvantage for sensitive species this may result in community change (Fleeger et al., 2003).

However, this remains open to discussion, as another explanation would be that the communities of the lightly contaminated streams may have not recovered from past impacts (Harding et al., 1998).

Overall, our investigation gives rise to the concern that the effect threshold for pesticides in the field is below a $\mathrm{TU}_{(D.\ magna)}$ of −2, which is currently regarded as protective, for example in the legislation on pesticides in the European Union (EEC, 1991).

5. Conclusions

This study showed that the structure and function of aquatic ecosystems may be impaired by pesticides. We suggest that effects may also occur below levels that are commonly thought to be protective. This highlights the importance of field studies since effects at these levels have not been observed in artificial systems. It is noteworthy that no effects were detectable in the Finnish study area under low pesticide usage.

A very important result for risk managers is that undisturbed upstream reaches improve the quality of impaired downstream reaches. This could constitute a valuable measure for future risk mitigation in addition to other innovations in agricultural practice.

Furthermore, current risk assessment would take a great step forward when implementing ecological knowledge. The use of biological traits in biomonitoring could be a starting point and may prove superior to taxonomically based approaches.

The trait-based SPEAR concept was capable of discriminating between the effects of pesticides and those of confounding factors and natural variation over large spatial scales. Thus, the results from the regional investigations may be extrapolated to other biogeographical regions in Central and North Europe. However, more studies are needed in non-European regions to assess the potential for extrapolation beyond Europe. For example, the concept could easily be applied to field observations from North America, as a database on invertebrate traits is available (Vieira et al., 2006).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.scitotenv.2007.04.040.

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