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Impact of triphenyltin acetate in microcosms simulating floodplain lakes. I. Influence of sediment quality

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Abstract Floodplain lakes in the Rhine–Meuse delta of the Netherlands vary considerably in levels of sediment-bound toxicants. Microcosm experiments were done to compare the ecological impact of the fungicide triphenyltin acetate (TPT) between test systems with clean or polluted sediments (10 microcosms each). Differences in sediment quality affected the structure of the aquatic communities that developed in the microcosms. Initially, a faster growth of the macrophyte *Elodea nuttallii* was observed on the polluted sediments, which contained not only toxicants but also higher organic matter and nutrient levels. Dynamics of TPT concentrations in the overlying water were very similar between the two types of test system. Higher levels of TPT, however, were found in the sediment compartment of the clean sediment systems containing a smaller macrophyte biomass. TPT was very persistent in the sediments. In both test systems representatives of several taxonomic groups showed clear responses to a single application of TPT, although benthic Nematoda were not affected. Although a few differences in the intensity and/or duration of TPT-related population responses were observed between the two types of test system, the background pollutants in the polluted sediment hardly affected the overall sensitivity of the aquatic community to the additional chemical stressor TPT.

Keywords Sediment quality · Community response · Additional stressor · Triphenyltin acetate · Microcosm

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Introduction

Populations and communities integrate the effects of environmental conditions over different spatio-temporal scales. These environmental conditions include natural and anthropogenic stress factors, and at the ecosystem level, several of them may act in concert. Although most experimental research has so far focused on the impact of individual stress factors, recent studies indicate that the effect of a particular chemical stressor may depend on the intensity of other stressors (Folt et al. 1999; Heugens et al. 2001; Van der Geest et al. 2002).

Multi-stress effects of pollutants are plausible in floodplain lakes of the Rhine–Meuse delta in the Netherlands. Sediments in these lakes differ in quality, with older sediments containing high concentrations of xenobiotic mixtures deposited in the 1960s and 1970s, while younger sediments are relatively clean (Beurskens et al. 1993). Although a large part of the sediment-bound toxicants is not directly bioavailable, these toxicants may still cause chronic stress due to a diffuse flux from sediment to (interstitial) water. In addition, organisms in floodplain lakes have to cope with occasional flooding and short-term exposures to chemicals like pesticides (Stäb et al. 1994; Liess and Schulz 1999). To date, it is not clear how the presence of sediment-bound pollutants affects the response of aquatic communities

to additional stressors. It cannot be excluded that the resilience of aquatic ecosystems against these additional chemical stressors is negatively impacted by sediment-bound 'background' pollutants. On the other hand, communities subject to 'background' pollutants may also be better adapted to cope with additional chemical stress. Our search of the open literature yielded no reports on experiments studying the impact of sediment-borne background pollutants on the sensitivity and resilience of aquatic communities subject to additional chemical stressors.

The objective of the present study was twofold: (1) to compare population and community responses (including recovery) between two types of aquatic microcosm, one containing clean and the other polluted sediment, after treatment with the additional stressor triphenyltin acetate (TPT), and (2) to experimentally investigate the ecological impact of TPT on the structure and functioning of freshwater ecosystems. We constructed microcosms containing clean or polluted sediments from river floodplain lakes, to which we added TPT as an additional stressor. The clean sediment was significantly less polluted with nutrients, metals, polyaromatic hydrocarbons (PAH), and polychlorobiphenyls (PCB) than the polluted sediment.

Ecosystem-level studies on the impact of organotin compounds in general, and of TPT in particular, are scarce. The present study is the first to be reported in the open literature that deals with the ecological impact of TPT in experimental freshwater ecosystems. The fungicide TPT is an organotin compound which is used to control a range of fungal diseases on a variety of crops (Grigarick et al. 1990) and was intensively used in potato crop farming in the Netherlands up to 2001. TPT compounds have also been applied as co-toxicants in some anti-fouling paints (Stäb et al. 1994). Organotins, including TPT, are highly toxic to a wide range of aquatic organisms (Fargasová 1998; Jak et al. 1998; Petersen and Gustavson 2000; Rehage et al. 2002). The most notorious and well-known compound is probably tributyltin (TBT), which was/is primarily used as an antifouling biocide on ships. In terms of the relative toxicity of all organotin compounds for aquatic organisms, TPT compounds and TBT are reported to be amongst the most toxic (Laughlin and Linden 1985; Vighi and Calamari 1985). Organotins degrade in the environment via biodegradation and photodegradation with halve-lives of 60–240 days (Loch 1990), but residence times for TPT and TBT in sediments of over ten years have also been reported (Fent et al. 1991).

The present paper focuses on the overall ecological impact of TPT in the two types of freshwater microcosm we constructed, with relatively clean and polluted sediments from river floodplain lakes. A second paper (Part II, Roessink et al. 2006) will compare the concentration–response relationships of aquatic populations in the TPT-stressed microcosms with the results of acute laboratory single species tests performed with TPT and a wide variety of freshwater species, including algae, macrophytes, zooplankters, and macroinvertebrates.

Materials and methods

Outdoor microcosms and experimental design

A total of 20 concrete outdoor microcosms (with a length of 140 cm, a width of 120 cm, and a depth of 80 cm) were used in the experiment. A sediment layer of approximately 10 cm and a water layer of approximately 50 cm were introduced in the test systems in November 2000, 8 months prior to the start of the experiment. The sediments originated from two lakes situated alongside the river Waal, in the Rhine catchment area. One of the lakes was polluted, while the other lake was considered to be clean (lakes coded DeO3B and DeO2, respectively in De Haas et al. 2002; Moermond and Koelmans 2002). The levels of inorganic and organic contaminants, as well as organic matter and nutrient (phosphorus) levels, were higher in the polluted sediment, while the greatest difference between the two was in PCB content (by a factor of 22) (see Table 1). Ten cosms were filled with polluted sediment (PS cosms) and 10 cosms with clean sediment (CS cosms).

After the sediments had been introduced, 20 shoots of the macrophyte *Elodea nuttallii* were planted in each microcosm, evenly distributed over the sediment compartment. The macroinvertebrate community in the test systems originated partly from individuals that were introduced via the sediment, but additional macroinvertebrates were also introduced 7 and 4 months before the start of the experiment, allowing enough time for the populations to acclimatize to the conditions in the test systems. The additional macroinvertebrates used as seeding material comprised taxa that are regularly found in shallow freshwater ecosystems in the Netherlands and were suspected to

Table 1 Sediment quality parameters of the two types of sediment used to construct the microcosms: Cd, Cu, Zn, ∑PAH in mg/kg dry weight, ∑PCB in µg/kg dry weight, OM (organic matter) in % of dry sediment, Total P in g/kg Data according to De Haas et al. (2002) and Moermond and Koelmans (2002)

	Clean (DeO2)	Polluted (DeO3b)
Cd	0.17	2.19
Cu	12	82
Zn	42	507
∑PAH	0.55	5.87
∑PCB	4.37	133.22
OM	2.7	9.0
Total P	0.4	1.5

be sensitive to TPT (e.g. Annelida, Tricladida, Mollusca). Phytoplankton and zooplankton species were not introduced as such but entered the cosms via the collected sediments, but also via the water used to fill the microcosms, which was collected from a freshwater reservoir present at the experimental facility.

The study was intended to end approximately 12 weeks after the TPT application. In the course of the experiment, several macroinvertebrate taxa died out in the microcosms, as a direct result of TPT application. These macroinvertebrates included Mollusca, Annelida and Turbellaria, taxa with not very highly developed abilities to recolonize isolated test systems. We therefore decided to prolong the study to investigate the possible recovery of invertebrate populations. To stimulate recovery of several macroinvertebrate taxa, they were deliberately reintroduced in low numbers in all microcosms at weeks 5, 19, and 34 after the TPT application (see Table 2). The second part of the experiment focused on the responses and recovery of zooplankton (additional sampling in week 23) and macroinvertebrates (additional sampling in weeks 23 and 40), since they comprised the most sensitive species.

Triphenyltin acetate application and analysis

In both types of test system (represented by 10 microcosms each), triphenyltin acetate was applied once as Fentin acetate Pestanal® (Sigma Aldrich Chemie BV, Zwijndrecht, The Netherlands) on June 18, 2001. The treatment regime followed a regression design with nominal TPT concentrations in the water column of 0, 1, 10, 30, and 100 µg/l (n=2). Treatment levels were derived from toxicity data available (Fargasová 1997; De Zwart 2002) and were randomly allocated to the cosms.

Table 2 Number of individuals of each taxon introduced in each cosm to facilitate potential recovery

Species	Week 5	Week 19	Week 34
Asellus aquaticus	10	10	20
Bythinia sp.	10		20
Erpobdella octoculata	10		
Gammarus pulex	10	10	8
Lumbriculus sp.	2		
Lymnaea stagnalis	10	5	2
Physa acuta		5	2
Pisidium sp.	10	10	
Planorbis contortus	5	10	
Planorbis corneus	10	5	
Planorbis planorbis	10	10	
Planorbis vortex/vorticulus		8	
Sphaerium sp.	3		
Turbellaria sp.	8	1	20
Valvata piscinalis	5		

Each cosm was treated by pouring 4 l of an application solution into the water and stirring gently with an iron rod to aid mixing, whilst taking care not to disturb the sediment layer or damage plants. In all microcosms, 0.42 ml 96% ethanol (methanol-free, 4% water) per liter water was used as a carrier solvent (0.04% v/v). Control cosms were treated with an equal amount of carrier solvent without TPT. Control cosms without carrier solvent were not present. To estimate initial concentrations, TPT was analyzed in subsamples taken from the solutions applied.

In the field, triphenyltin acetate is quickly converted to triphenyltin hydroxide (Loch 1990). Since our analysis of water and sediment samples was unable to distinguish between the acetate and hydroxide form, we refer to both as TPT in presenting data on the fate of the test compound. Water samples to study the fate of TPT were only taken from the microcosms treated with 10 and 30 µg/l. This was done at 3 and 10 h and at 1, 3, 7, 14, and 28 days after TPT application. Depth-integrated water samples were taken using a Perspex tube (Ø 4 cm, length 50 cm), and on each sampling date two samples were collected from each cosm and combined. Approximately 500 ml of each combined sample was stored in a glass bottle and taken to the laboratory for further analysis. In the laboratory, 100 ml was transferred to a 250 ml Schott bottle (Louwers-Hapert, Hapert, The Netherlands) and used for further TPT analysis.

Sediment samples were taken at weeks 2, 4, 15, 25, and 40 after TPT application. Sediment was sampled using a Perspex tube (Ø 4 cm, length 50 cm), and two samples per sampling date were collected from the controls and the 30 µg/l cosms. The water phase was removed and the cores were stored in a freezer at <-20°C until analysis. The top 5 cm of each sediment core was transferred to a glass centrifuge tube (Ø 4.8 cm, content 240 cm^3) and thawed overnight. The next day, the cores were mixed with a rod for 0.5 min and centrifuged at 2656 g. Pore water was collected in a glass bottle and the remaining sediment was stored at 5°C. To analyze the pore water, 5 ml sodium acetate buffer (pH=5), a known amount of hexane, and 100 µl 2% sodium tetraethylborate and ethanol were added and the mixture was shaken for 15 min at 0.52 g. A known amount of the hexane was evaporated to 1 ml and transferred to a GC vial. Extraction efficiency was determined by adding 0.05 or 0.25 ml of a 100 µg/ml TPT solution to 25 ml pore water and treating these solutions as samples. The extraction efficiency in pore water was tested by spiking pore water from the controls with a known amount of TPT in ethanol. The recovery was found to be 133% (n=6; SD=23%); no corrections were made for this efficiency.

After 5 days, the stored sediments were transferred to aluminum containers and mixed. A third remained in the

container for dry weight analysis, a third was transferred to a Schott bottle and weighed for further TPT analysis, and a third was stored at < -10°C. Dry weight was analyzed by drying the samples overnight at 105°C. A solution of 37% HCl, 48% HBr, and deionized water (1:1:0.4 v/v/v) was added to the Schott bottle, after which the bottle was shaken (standing up) for 30 min at 0.52 *g*. A quantity of 80 ml hexane was added and the samples were extracted by shaking for 60 min at 0.52 *g*. The hexane layer was transferred to a 250 ml Schott bottle, after which 25 ml water, 5 ml NaAc buffer (pH=5), and 100 µl 2% sodium tetraethylborate were added, and the samples were shaken for 15 min at 175 rpm. The hexane layer was concentrated and transferred to a GC vial and analyzed. Extraction efficiency was analyzed using remaining sediment from control cosms. Amounts of 25–34 g sediment were weighed into a Schott bottle, and 250 µl of a 100 or 1 µg/ml stock solution was added. After 15 min, they were extracted and analyzed as described above. The extraction efficiency for sediments was tested by spiking blank sediment samples with a known amount of TPT in ethanol. The recovery was found to be 88% (n=4; SD=18%); no corrections were made for this efficiency.

Organotin analysis followed the methods described in the Dutch guidelines (NVN5729 2001) and ethylated organotins were detected on GC-MSD in Selective Ion Mode (GC: HP 6890 with auto injector HP 7683; MSD: HP 5973 Network MSD, Hewlett Packard, Palo Alto, USA). 2 ml buffer solution (pH=5; 120 g HAc+272 g NaAc per liter), 100 µl 2% sodium tetraethylborate and 20 ml hexane were added. The water phase was extracted by shaking for 15 min at 0.52 *g*. Part of the hexane layer was removed and transferred to a GC vial. The limit of detection of TPT in water was 1 µg/l. The recovery of the extraction procedure was tested by spiking blank water samples with a known amount of TPT in ethanol. The recovery was found to be 92.7% (n=4; SD=12.7%). Because sediment and water recoveries fell within the measurement error of the GC–MS, no corrections were made for these recoveries.

Water quality

Dissolved oxygen (DO), pH, conductivity, and alkalinity were measured at −4, −3, −2, −1, 0.4, 1, 2, 4, 6, 8, 12, 23, and 40 weeks after TPT application. DO was measured with a WTW Oxi330 oxygen meter and oxygen probe at a depth of 10 cm. Conductivity and pH were measured with a WTW LF 191 conductivity meter and a WTW PH197 pH meter, respectively. Alkalinity was measured in 100 ml samples taken at a depth of 10 cm (titration with 0.05 N HCl until pH 4.2; WTW PH197 pH meter).

Macroinvertebrates

Macroinvertebrates were sampled from each microcosm at −4, −2, 0.4, 2, 4, 8, 12, 23, and 40 weeks after TPT application by means of artificial substrates, viz., multiplates and pebble baskets. In each system, two multiplates and two pebble baskets were incubated. Details of the substrates used have been described by Brock and coworkers (Brock et al. 1992).

On each sampling day, the artificial substrates were gently retrieved from the microcosm using a net to prevent organisms escaping. Pebble baskets were first washed in a container to remove invertebrates from the pebbles. Subsequently, the macroinvertebrates present on both substrates were sorted by hand, identified, counted alive, and then returned to the microcosm. Data from artificial substrates was pooled for further analysis.

Zooplankton

Zooplankton was sampled from each cosm in weeks −4, −2, −1, 0.4, 1, 2, 4, 6, 8, 12, and 23, using a Perspex tube (length: 0.4 m; volume: 0.8 l). Several subsamples were collected, evenly distributed over the cosms, until a 5 l sample had been obtained. The 5 l sample was concentrated by means of a plankton net (Hydrobios, Kiel, Germany; mesh size: 55 µm) and was preserved with formaldehyde (final concentration 4%). Cladocera and Rotifera were counted and identified to the lowest possible taxon. Copepods were counted and classified into calanoids and cyclopoids. Cladocera and copepods were counted and identified using a binocular microscope, while rotifers were counted and identified using an inverted microscope. Abundances were adjusted to numbers of organisms per liter.

Sediment dwelling Nematoda

Nematode samples were taken with the same Perspex tubes used to sample sediment for TPT analysis. On each sampling date, two sediment cores were collected from each microcosm and the top 5 cm layers of these cores were pooled for analysis. Samples were immediately preserved with formalin (final concentration approximately 4%). Sampling took place at −3, 2, and 12 weeks after TPT application. Total numbers of nematodes in the samples were determined by counting under a stereo-microscope. For identification, 100–200 specimens (in some cases the whole sample) were mounted on slides and observed under a light microscope at a magnification of 630×. Occasionally, magnification of 1000× (oil) was used. Identification to species level was not always possible, due to the absence of adult specimens. In these cases, genus or family names are given.

Primary producers

Water samples to assess phytoplankton species composition and phytoplankton chlorophyll-*a* concentrations were taken in weeks −2, −1, 0.1, 1, 2, 4, 6, 8, and 10. In addition, phytoplankton chlorophyll-*a* samples were taken at 15 and 23 weeks after TPT application. Several depth-integrated water samples were collected using a Perspex tube (length: 0.4 m; volume: 0.8 l) until a total sample of 5 l had been obtained. From the 5 l sample, a 1-l subsample was taken for chlorophyll-*a* analysis, which was concentrated using a glass-fiber filter (Schleicher and Schuell GF52, mesh size: 1.2 μm). Filters were inserted in Petri dishes, wrapped in aluminum foil, and stored at −20°C prior to analysis. Pigments were analyzed using a spectrophotometer (Beckman, DU-64) following the method described by Moed and Hallegraeff (1978). Another 1-l subsample was preserved with Lugol and formalin (final concentration 4%) and used to identify the phytoplankton. In general, phytoplankton was counted and identified to genus level, using an inverted microscope.

The percentage cover of macrophytes (including filamentous algae) was estimated at −2, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, and 13 weeks after TPT application.

Decomposition of Populus leaves

Decomposition of particulate organic matter (POM) was studied by means of the litter bag technique (Brock et al. 1982). The POM used consisted of *Populus* × canadensis leaves. The *Populus* leaves had been leached three times for 2 days to remove soluble humic compounds. To allow this material to be stored, it was dried in an oven for 72 h at 60°C. In the decomposition assessments, 2 g dry weight of *Populus* leaves were enclosed in each litter bag, consisting of a sieve of stainless steel wire (mesh size: 0.7×0.7 mm^2). In each microcosm, two litter bags were incubated at mid-depth in the water column for a period of 2 weeks. Whenever a set of litter bags was retrieved on a sampling day, a new set was incubated. The organic plant material was dried in aluminum foil at a temperature of 105°C. After 24 h, dry weight was determined. The decomposition over a 2-week period was expressed as % remaining organic material.

Data analysis

Prior to analysis, the macroinvertebrate and nematode data was $\ln(2x+1)$ transformed, where x is the abundance value. For zooplankton and phytoplankton, data was transformed by $\ln(10x+1)$ and $\ln(0.001x+1)$, respectively. This was done to down-weight high abundance values and approximates a log-normal distribution of the data (Van den Brink et al. 1995). No Observed Effect Concentrations (NOECs) at parameter or taxon level were calculated using the Williams test (ANOVA) (Williams 1972). This test assumes that the mean response of the variable is a monotonic function of the treatment, thus leading to the expectation of increasing effects with increasing dose. The analyses were performed with the Community Analysis computer program (Hommen et al. 1994), resulting in a summary of NOECs in each sampling week for the data analyzed. The threshold level for P was 0.05 for all statistical analyses.

The effects of the TPT treatment at the community level (macroinvertebrates, zooplankton, nematodes, phytoplankton) were analyzed by the Principal Response Curves method (PRC), which is based on the Redundancy Analysis ordination technique, the constrained form of Principal Component Analysis (Van den Brink and Ter Braak 1999). The PRC method yields a diagram showing the differences between treatments and controls. A full description and discussion of the PRC method has been provided by Van den Brink and Ter Braak (1997, 1998, 1999). The PRC analysis was performed using the CANOCO software package, version 4.02 (Ter Braak and Smilauer 2002). The results of the PRC analysis can also be evaluated in terms of the fractions of the variance explained by the factors time and treatment, and the PRC diagram shows the fraction of the variance that is explained by treatment. In the CANOCO computer program, Redundancy Analysis is accompanied by Monte Carlo permutation tests to assess the statistical significance of the effects of the explanatory variables on the species composition of the samples (Verdonschot and Ter Braak 1994; Van der Brink et al. 1996).

The significance of the PRC diagrams in terms of displayed treatment variance was tested by Monte Carlo permutation of the microcosms, i.e., by permuting whole time series of microcosms in the partial redundancy analysis from which PRC is obtained, using an F-type test statistic based on the eigenvalue of the component (Van der Brink and Ter Braak 1999).

Monte Carlo permutation tests were also performed for each sampling date, using the ln-transformed nominal doses as the explanatory variable (Van der Brink et al. 1996). This allowed the significance of the treatment regime to be tested for each sampling date. If a significant relation between treatment regime and species composition was found, we also determined which treatment levels differed significantly from the controls, so as to infer the No Observed Effect Concentration at the community level ($NOEC_{community}$). $NOEC_{community}$ was calculated by applying the Williams test (Van den Brink et al. 1996; Van den Brink and Ter Braak 1999).

For the nematode community, a Maturity Index (MI) was calculated according to the following formula:

$$\mathrm{MI} = \sum_{i=1}^{s} v_i * p_i \quad (1)$$

where v_i is the colonizer/persister value of taxon i and p_i is the proportion of taxon i in relation to the total number of non-plant feeding nematodes. The MI is an ecological index for nematodes based on the colonizing or persisting characteristics of nematode families, and divides the nematodes over five classes on a colonizer/persister scale. Nematodes can be placed on this arbitrary c/p scale ranging from 1 (for extreme colonizers, such as *Monhysteridae*, which have generation times of only a few days) to 5 (for extreme persisters, such as *Enoplidae*, which have a generation time of one year). Plant-feeding nematodes and dauerlarvae are not taken into account (Bongers 1990; Bongers and Bongers 1998).

Results

Fate of TPT

Concentrations of TPT in the water column of the microcosms, based on measured concentrations in the solutions applied to the test systems, varied between 95 and 102% of the intended nominal concentrations (see Table 3). Figure 1 shows that 3 h after application, the measured peak concentrations in the water column were higher than the intended concentrations, viz., 35 and 13 vs. 30 and 10 μg/l and 45 and 16 vs. 30 and 10 μg/l for the CS and PS systems, respectively. This can be explained by incomplete mixing during the first hours after application, particularly in the presence of higher densities of macrophytes (PS systems). Nevertheless, the overall dissipation rate of TPT in the water column was similar in both types of test system (Figs. 1, 2).

On all sampling dates, total TPT levels in the sediment were somewhat higher in the CS cosms than in the PS cosms (Fig. 2), despite the fact that the sediment of the PS cosms had a higher organic matter content (Table 1). The reason for this might be the smaller macrophyte biomass in the CS test systems at the time of TPT application (see also Fig. 11). Most probably, a larger proportion of the TPT dose was sorbed to the larger stock of macrophytes in the PS systems.

Concentrations of TPT in pore water remained much lower that those initially measured in the overlying water. In both types of microcosm, however, the rates of disappearance of TPT from the pore water and total sediment compartments were considerably lower than those initially measured in the overlying water (Fig. 2). TPT thus appeared to be very persistent in the sediment compartment.

Table 3 Percentages (mean and standard deviations) of calculated initial concentrations of triphenyltin acetate (TPT) in the overlying water of the microcosms relative to intended concentrations

CS cosms intended TPT (μg/l)	% of intended mean	SD	PS cosms intended TPT (μg/l)	% of intended mean	SD
1	97.4	13.1	1	104.4	30.6
10	88.7	22.7	10	99.5	3.4
30	97.6	14.3	30	99.7	7.7
100	96.7	6.0	100	105.4	5.6

Water quality

Dissolved oxygen concentration dynamics differed considerably between the CS and PS cosms (Fig. 3). In the CS cosms (Fig. 3A), DO levels were already steeply declining before TPT application. This decline in the pre-treatment period can be explained by the natural collapse (and accompanying degradation processes) of filamentous algae that initially dominated the vegetation in the CS systems (see also Fig. 11C, D). This pre-treatment decline in DO levels obscured possible treatment-related effects in terms of this endpoint (Table 4). Hardly any filamentous algae were present in the PS cosms, and DO levels here started to decline immediately after TPT application (Fig. 3B). The decline in DO concentrations in the PS cosms might be partly explained by the use of ethanol as a carrier solvent, since DO levels in the controls (which received no TPT but only the carrier solvent) dropped from approximately 12 to 6 mg/l during the first two weeks after application (Fig. 3B). The ethanol was apparently a readily available energy source for microorganisms, which most probably consumed large quantities of DO immediately after ethanol application. Nevertheless, a clear concentration–response pattern in DO concentrations was also observed in the PS cosms after TPT application (Fig. 3B). Relative to controls, statistically significant declines in DO levels were observed during the first two weeks after TPT application, with an NOEC as low as 1 μg TPT/l (Table 5). Note that all mentioned calculated NOEC values in this paper are based on initial nominal concentrations. In all CS and PS cosms, DO concentrations increased again to levels well above 5–6 mg/l between 2 and 4 weeks after treatment.

The treatment-related decline in DO levels in the PS cosms in particular can be explained by the photosynthesis-inhibiting properties of TPT (Marsot et al. 1995; Mooney and Patching 1995). This is in line with the observed treatment-related pH responses (Fig. 3D; Table 5), conductivity (Table 5), and alkalinity (Table 5) in the PS cosms during the first weeks after TPT application: conductivity and alkalinity temporarily increased, while pH

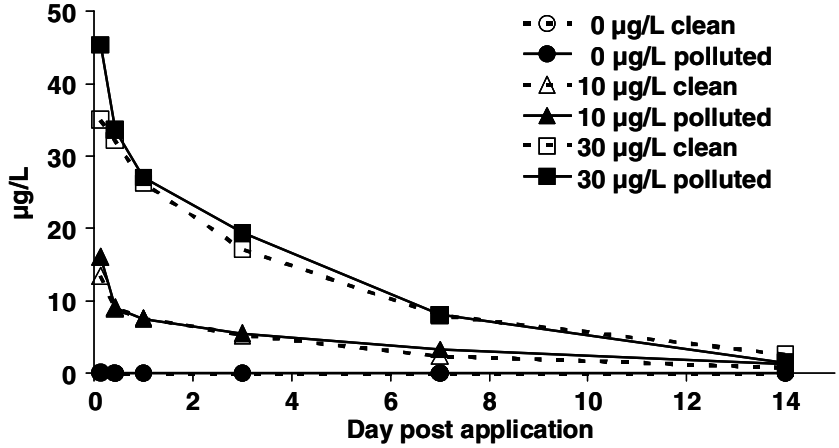

Fig. 1 Dynamics of TPT concentrations (geometric mean) in the water phase for the control, 10, and 30 µg/l treatments in the CS (clean sediment) and PS (polluted sediment) cosms

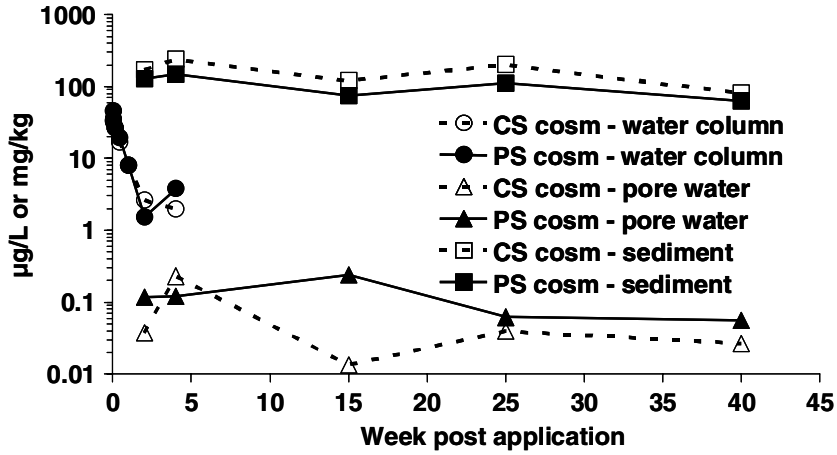

Fig. 2 Dynamics of TPT concentrations (geometric mean) in the water column (µg l^{-1}), pore water (µg/l), and sediment (µg/kg dry weight) for the 30 µg/l treatment in cosms with clean (CS) and polluted (PS) sediment

and DO temporarily decreased. This response is consistent with a decrease in photosynthesis and is know as the DO-pH-Alkalinity-Conductivity Syndrome (Brock et al. 1993).

Responses of macroinvertebrates

A total of 83 different macroinvertebrate taxa were identified in the cosms, of which 82 and 77 were present in the CS and PS systems, respectively. Although there was a large overlap in species composition between system types, greater differences existed in relative dominance of taxa. The community of the CS systems showed greater abundance of Coleoptera, *Stylaria lacustris* (Oligochaeta), Ostracoda, Pisididae, and the snails *Valvata* sp. and *Planorbis planorbis*, while *Polycelis nigra/tenuis* (Tricaldida), *Cloeon dipterum* (Ephemeroptera), *Chaoborus obscuripes* (Diptera), *Asellus* sp. (Crustacea), *Erpobdella octoculata* (Hirudinea), and *Physa fontinalis* (Mollusca) were more common in the PS systems.

Multivariate analysis of the macroinvertebrate community sampled in the CS and PS microcosms revealed clear treatment-related effects in both types of test system, which can be ascribed to the treatment with TPT (Fig. 4; Table 6). For instance, Fig. 4A indicates that, compared to the controls, the largest deviations in species composition of macroinvertebrates occurred in the 100 and 30 µg/l cosms, while smaller deviations were found in the 10 µg/l cosms. The species weight (b_k) shown on the right-hand side of the diagram can be interpreted as the affinity of each species with the response in the diagram. Thus *Stylaria lacustris*, which has the highest positive species weight, had the greatest decrease at the higher treatment levels. The negative weight of *Chironomidae* sp. in the diagram indicates that its numbers increased at the higher

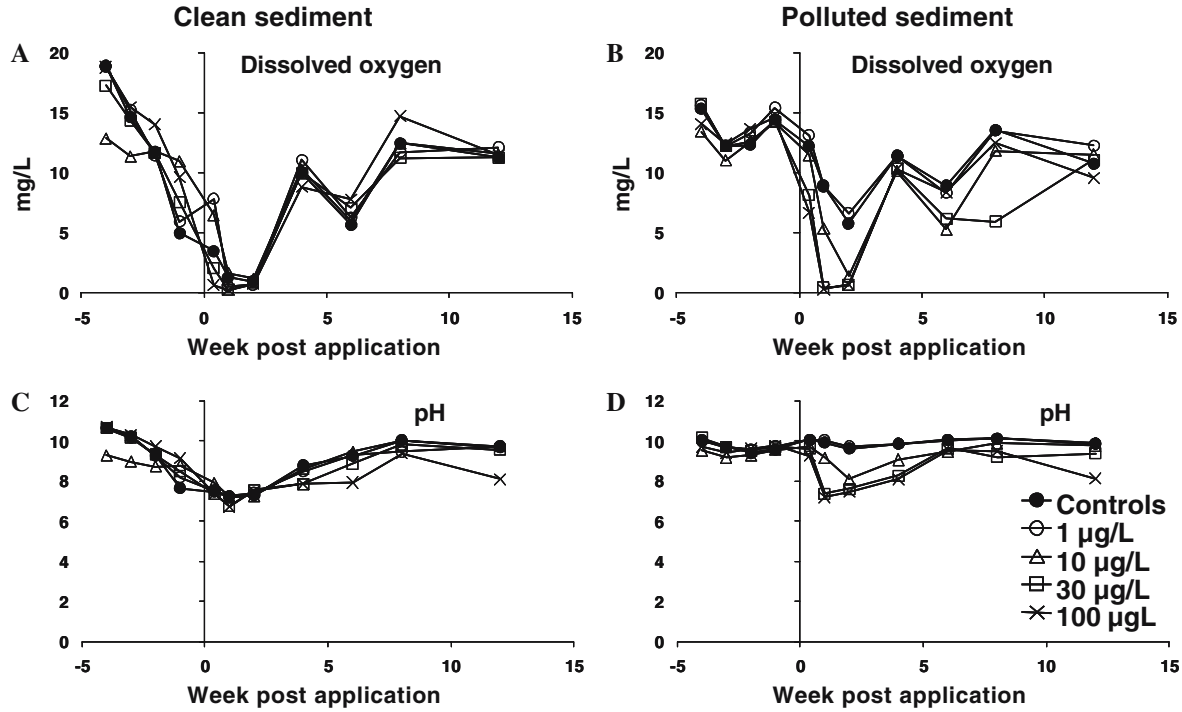

Fig. 3 Dynamics of Dissolved Oxygen (**A** and **B**) and pH (**C** and **D**) in clean sediment and polluted sediment cosms treated with TPT, during the first 12 weeks after application. Calculated NOEC values are presented in Tables 4 and 5

treatment levels, relative to controls. Most taxa had a positive score on the B_k axis and consequently showed an overall treatment-related decrease in abundance. A limited number of taxa had a negative B_k score and thus showed an overall increase with increasing TPT concentrations.

The weaker initial response in the CS systems compared with the PS microcosms may be explained by the oxygen stress observed in all CS systems due to the natural collapse of filamentous algae around the time of TPT application (Fig. 4) and the application of ethanol as a solvent. Nevertheless, for both the CS and PS microcosms, Monte Carlo permutation tests revealed significant differences on all post-treatment sampling dates (Table 6). Both the CS and PS microcosms had an $NOEC_{community} < 1$ µg/l on one or two isolated sampling dates. On the basis of the response observed on at least two consecutive sampling dates, the overall critical threshold level was 1 µg TPT/l in both types of test system (Fig. 4, Table 6).

A comparison of the different response curves presented in Fig. 4 shows that recovery of the macroinvertebrate community seemed to be faster in the systems with polluted sediments (PS). However, at the end of the experimental period, an $NOEC_{community}$ of 10 µg/l was calculated for both the CS and PS systems (Table 6), indicating the occurrence of long-term effects after a single application of TPT. This is remarkable, since after approximately 2–3 weeks, TPT concentrations in the water column had dropped to approximately 1 µg/l in the systems treated with 30 µg/l (Fig. 1). In the 30 µg/l cosms, significant effects on the macroinvertebrate community were still observed 42 weeks after TPT application, despite the introduction of small numbers of sensitive taxa in weeks 5, 19, and 34 to facilitate potential recovery (Table 2).

Univariate analysis of the macroinvertebrates sampled in the CS and PS microcosms also revealed clear treatment-related effects in both types of test system, which can be ascribed to the treatment with TPT (Tables 4 and 5; Figs. 5, 6). Representatives of Annelida, Tricladida and Mollusca in particular showed clear treatment-related declines in both the CS and PS systems, with overall NOECs in the range of < 1–10 µg/l. The most sensitive macroinvertebrate taxa in the CS systems included the oligochaete worm *Stylaria lacustris* and bivalve mollusks, for which NOECs < 1 µg/l were calculated on at least two consecutive sampling dates (Table 4). In the PS systems, consistent NOECs < 1 µg/l were calculated for *Stylaria lacustris* and the triclad *Polycelis nigra/tenuis*, while the snails *Planorbis contortus* and *Physa acuta* showed NOECs < 1 µg/l on two individual sampling dates (Table 5). Of these taxa bivalve mollusks and *P. contortus* were reintroduced to the cosms at week 5 and 19, while *Turbellaria* sp. were also reintroduced at week 34. And *P. acuta* was only reintroduced at week 19 and 34 (Table 2).

Table 4 Univariate analysis of the treatment-related responses of water quality and population endpoints in clean sediment cosms, using the Williams test ($P \leq 0.05$)

Taxon	−4	−3	−2	−1	0.4	1	2	3	4	5	6	7	8	10	12	13	16	23	42
Water quality																			
DO	>		>	>	1 (↑)	>	>	>	30		>		30 (↑)	>	>			1 (↑)	>
pH	>		>	>	30 (↑)	>	10	>	>		30		>	>	30			>	10 (↑)
Conductivity	>		>	>	30	>	>	>	10 (↑)		1 (↑)		>	>	30 (↑)			>	>
Alkalinity	>		>	>	1	>	>	>	10 (↑)		30 (↑)		>	>	30 (↑)			>	>
Macroinvertebrates																			
Annelida	>			>		>	< 1		1				1		10			30	10
Glossiphonia complanata	>			30 (↑)		>	>		>				30		30			1	10
Helobdella stagnalis	>			>		>	10		>				1		10			1	30
Oligochaeta	>			>		1	< 1		30				>		>			>	n.p.
Stylaria lacustris	>			>		10	< 1		< 1				1		10			< 1	>
Tricladida	>			>		>	>		>				10		1			1	>
Polycelis nigra/tenuis	>			>		>	>		>				10		1			1	>
Mollusca	>			>		>	>		1				10		1			1	1
Planorbis contortis	< 1 (↑)			>		>	>		1				30		1			1	1
Planorbis planorbis	>			>		30	10		< 1				10		1			10	1
Radix peregra	>			>		10	>		1				>		1			1	>
Physa acuta	>			< 1		>	>		>				>		< 1			>	1
Bivalvia	>			>		>	>		< 1				1		1			< 1	< 1
Arthropoda	>			>		30	>		>				>		>			30	30
Asellidae	>			>		30	>		10				>		>			>	>
Chaoborus obscuripes	>			>		>	>		>				10		>			10	10
Chironomidae	>			>		>	30		>				1 (↑)		>			>	10 (↑)
Cloeon dipterum	>			>		>	>		>				30		>			30 (↑)	>
Dytiscidae larvae	>			>		>	>		>				>		>			30 (↑)	30 (↑)
Zooplankton																			
Cladocera	>			>	>	30	30	>	>		30		30		30			>	
Chydorus sphaericus	>			>	>	>	< 1	>	>		>		10		30			>	
Graptoleberis testudinaria	>			>	>	>	>	>	>		>		>		10			10	
Simocephalus vetulus	>			>	< 1	>	>	>	>		>		30		>			30	
Copepoda	>			>	>	10	10	1	1		1		1		10			10	
Copepoida	>			>	>	1	1	1	1		>		1		>			10	
Cyclopoida	>			>	30	>	10	1	10		1		1		10			10	
Amoeba	10 (↑)			>	>	>	>	>	>		1 (↑)		30		>			>	
Rotifera	>			>	>	>	>	30	10		10		>		1			>	
Keratella quadrata	>			>	>	10	< 1	< 1	>		>		>		>			>	
Primary producers																			
Phytoplankton chl-*a*				>	>	>	30 (↑)	30 (↑)	>		>		10 (↑)	>			>	>	
Cocconeis				>	>	>	>	>	>		10 (↑↓)		< 1 (↑↓)	30 (↑↓)					
Phacus				>	>	>	>	< 1 (↑↓)	< 1 (↑↓)		>		>	>					
Trachelomonas				>	>	< 1 (↑↓)	>	< 1 (↑↓)	< 1 (↑↓)		< 1 (↑↓)		>	10 (↑↓)					
Scenedesmus				10	>	< 1	30	>	>		>		>	>					
% cover *Elodea*				>		>	>	30	30	30	30	30	30	30	30	30			

The No Observed Effect Concentration (NOEC) of each endpoint is given per sampling week. Only those endpoints that showed a significant response on two consecutive dates or on three non-connected sampling dates are presented

> indicates an NOEC of >100 μg/l; empty entries indicate that no sampling occurred for these endpoints on these dates; n.p. indicates that the Williams test could not be performed due to lack of specimens in several cosms; (↑) indicates an increase instead of a decrease; (↑↓) significantly different (Williams test) but with clear non-linear response

Compared with other taxonomic groups, the Arthropoda as a whole seemed to be less sensitive to TPT application (Figs. 5G, H). However, relatively large differences in responses were observed between taxa within the group of Arthropoda (Tables 4 and 5). For example, in both the CS and PS cosms, larvae of the phantom midge *Chaoborus obscuripes* were relatively sensitive to TPT application, without any signs of recovery (Fig. 6A, B); the CS (Table 4) and PS (Table 5) cosms showed NOECs of 10 and 1 μg/l, respectively. In contrast, Asellidae showed a short-term treatment-related decline, with complete recovery on the last two sampling dates (Fig. 6C, D). A similar response pattern was observed for larvae of the ephemeropteran *Cloeon dipterum*, but this species even showed a significant increase in densities at the highest treatment level, relative to controls, after the initial decline (Fig. 6E, F, Table 5). Taxa profiting from the TPT application were predominantly some arthropods. For example, Culicidae

Table 5 Univariate analysis of the treatment-related responses of water quality and population endpoints in the polluted sediment cosms, using the Williams test ($P \leq 0.05$)

Taxon	−4	−3	−2	−1	0.4	1	2	3	4	5	6	7	8	10	12	13	16	23	42
Water quality																			
DO	>	>	30 (↑)	>	10	1	1		>		>		10		>			>	>
pH	>	>	>	>	10	1	1		1		1		10		10			30	>
Conductivity	>	>	>	>	10	10 (↑)	1 (↑)		30 (↑)		1		>		30 (↑)			>	>
Alkalinity	>	>	>	>	>	>	>		30 (↑)		1		>		10 (↑)			30 (↑)	>
Macroinvertebrates																			
Annelida	>		>		>		10		10				1		10			10	10
Erpobdella sp.	n.p.		>		10		>		1				< 1		>			>	>
Erpobdella octoculata	>		>		30		< 1		>				10		1			1	< 1
Glossiphonia complanata	>		>		>		>		>				10		30			1	10
Helobdella stagnalis	>		>		>		10		10				1		10			< 1	>
Stylaria lacustris	>		>		< 1		>		< 1				< 1		< 1			>	n.p.
Tricladida	>		>		30		< 1		< 1				< 1		10			10	10
Polycelis nigra/tenuis	>		>		30		< 1		< 1				< 1		10			10	10
Mollusca	>		30		30		1		1				1		1			>	>
Planorbis contortus	>		>		>		< 1		1				< 1		>			>	>
Physa acuta	30 (↑)		>		30		1		< 1				1		< 1			>	>
Physa juvenile	>		30 (↑)		10		< 1		>				>		>			>	>
Physa fontinalis	>		>		>		1		1				>		>			>	>
Arthropoda	>		>		30		>		< 1				>		< 1			1	>
Asellidae	>		>		30		10		10				30		30			>	>
Chaoborus obscuripes	>		>		>		10		1				>		1			1	1
Chironomidae	>		>		30		>		>				1 (↑)		1 (↑)			>	>
Cloeon dipterum	>		>		>		1		1				30		1 (↑)			30 (↑)	30 (↑)
Culicidae	n.p.		>		n.p.		30 (↑)		1 (↑)				>		>			n.p.	n.p.
Tabanidae	>		>		>		1		1				< 1		>			n.p.	n.p.
Corixidae juvenile	>		>		>		>		30				10 (↑)		30 (↑)			>	>
Zooplankton																			
Cladocera	>		>	30	30	10	10		10		30		>		>			10	
Chydorus sphaericus	>		>	>	>	30	10		10		10		>		>			>	
Graptoleberis testudinaria	>		>	>	>	>	30		1		1		10		10			>	
Simocephalus vetulus	>		>	>	>	1	1		10		10		>		>			>	
Copepoda	>		>	>	10	1	1		1		10		>		30			>	
Copepoida	>		>	>	>	1	>		30		10		10		>			>	
Cyclopoida	>		>	>	10	1	1		1		1		>		>			>	
Rotifera	>		>	>	>	30	30		10		30		10		30			30	
Keratella quadrata	>		>	>	10	1	1		< 1		>		>		>			>	
Lepadella patella	< 1		>	>	>	>	>		1		1		30		>			< 1	
Primary producers																			
Phytoplankton chl-*a*	>		>	>	1	< 1	10 (↑)		>		10 (↑)		30 (↑)	>			1	>	
Cosmarium	>		>	>	>	>	>		>		30 (↑)		30 (↑)	30 (↑)					
% cover *Elodea*				>	>	>	>	30	30	30	10	10	10	30	30	10			

The No Observed Effect Concentration (NOEC) of each endpoint is given per sampling week. Only those endpoints that showed a significant response on two consecutive dates or on three non-connected sampling dates are presented

> indicates an NOEC of >100 μg/l; empty entries indicate that no sampling occurred for these endpoints on these dates; n.p. indicates that the Williams test could not be performed due to lack of specimens in the cosms; (↑) indicates an increase instead of a decrease

(Fig. 6G, H), Dytiscidae larvae (Table 4), and Chironomidae (Table 5) showed a temporary treatment-related increase in population densities, indicating the occurrence of indirect effects.

Responses of zooplankton

A total of 86 zooplankton taxa were identified, 57 of which occurred in the CS cosms and 76 in the PS systems. The cladocerans *Peracantha truncata*, *Disparalona rostrata*, and *Graptoleberis testudinaria*, and the rotifers *Hexarthra mira*, *Euchlanis lyra*, and *Conochilus* sp were more common in the CS cosms, while cyclopoids, the cladoceran *Simocephalus vetulus* and the rotifers *Keratella quadrata*, *Lecane lunaris* and *Lepadella patella* were more abundant in the PS cosms.

Multivariate analysis of the zooplankton community sampled in both types of test system (CS and PS) showed a clear treatment–response relationship (Fig. 7). On the B_k axis on the right side of the graphs, it can be seen again that

Table 6 Significance of the Monte-Carlo permutation tests and $NOEC_{community}$ values calculated from the PRC analysis of the macroinvertebrate communities in the TPT treated microcosms with clean (CS) and polluted (PS) sediment

Week relative to application	CS cosms		PS cosms	
	P-value	$NOEC_{community}$ (μg/l)	*P*-value	$NOEC_{community}$ (μg/l)
−4	>0.05	>100	>0.05	>100
−2	>0.05	>100	>0.05	>100
0.4	0.015	>100	0.012	10
2	0.001	1	0.001	1
4	0.004	<1	0.001	1
8	0.001	1	0.001	<1
12	0.001	1	0.001	1
23	0.001	1	0.001	<1
40	0.001	10	0.010	10

most taxa had positive scores and thus showed an overall treatment-related decrease in abundance. A limited number of taxa had negative B_k scores and thus showed an overall increase with increasing TPT concentrations. Again, the more pronounced DO decrease in the CS systems (due to a natural decline in filamentous algae) most probably obscured the response pattern of the zooplankton community of the CS systems during the first weeks after TPT application, compared with that of the PS cosms. Nevertheless, in both types of test system, Monte-Carlo permutation tests revealed significant treatment-related effects during the whole post-treatment period, except for weeks 0.4 and 2 in the CS systems and week 12 in the PS cosms (Table 7). In both types of test system, an $NOEC_{community}$ of 1 μg TPT/l was calculated for zooplankton on at least two consecutive sampling dates (Table 7). On the last sampling date (week 23), the $NOEC_{community}$ calculated for the CS systems was 10 μg/l, while that for PS cosms was 30 μg/l. This suggests a somewhat faster recovery of the zooplankton community in the PS systems.

The three main zooplankton groups (Copepoda, Cladocera, and Rotifera) showed clear treatment-related responses (Fig. 8); overall, Copepoda were the most sensitive, with consistent NOECs as low as 1 μg TPT/l (Tables 4 and 5). The most sensitive zooplankton population in the CS microcosms, however, was that of the rotifer *Keratella quadrata*, with an NOEC < 1 μg/l on two consecutive sampling dates during the first weeks after TPT application (Table 4). This zooplankton species showed a fast recovery, in contrast to initially less sensitive taxa like Cyclopoida and *Graptoleberis testudinaria*, which still had an NOEC of 10 μg/l on the last sampling date (Table 4). The most sensitive zooplankton populations in the PS microcosms were *Graptoleberis testudinaria*, *Simocephalus vetulus*, Cyclopoida, *Keratella quadrata*, and *Lepadella patella*, all with a consistent lowest NOEC of 1 μg/l (Table 5). Of these taxa, *Simocephalus vetulus*, Cyclopoida, and *Keratella quadrata* showed a relatively fast recovery in which significant differences could not be calculated anymore after 8 weeks post-application.

Responses of sediment-dwelling nematodes

A total of 44 nematode taxa were identified in sediment samples from the cosms, 31 of which were found in the clean and 36 in the polluted sediment systems. Some characteristic taxa in the community of the CS cosms were *Tobrilus* sp., *Monhystera* sp., *Monhystera riemanni*, and *Mononchus aquaticus*. Taxa that were more abundant in the community of the PS cosms were *Eumonhystera similis*, *Daptonema dubius*, and *Etmolaimus pratensis*.

Multivariate analysis of the nematode community, followed by Monte-Carlo permutation testing, revealed that the nematode community structure differed significantly between the CS and PS microcosms. Nevertheless, no TPT-related effects on nematode community structure were found in either of the two types of test system. In addition, calculating and testing the Maturity Index of the nematode community sampled in the two types of test system did not reveal significant treatment-related effects. The calculated Maturity Index values were all in the same range (2.04–3.00; see Table 8). This relatively low range indicates that the nematode communities in both the CS and PS microcosms include mainly colonizers, characterized by high reproduction (Bongers 1999).

Response of primary producers

A total of 45 different phytoplankton taxa were identified, 43 of which were found in the CS microcosms and 40 in the PS test systems. Overall, the CS cosms were characterized by a higher abundance of *Cryptomonas*, *Tetraedon*, and *Monorhaphidium*, while *Anabaena*, *Epithemia*, and *Cocconeis* were more abundant in the PS cosms.

In both the CS and PS test systems, multivariate analysis of the phytoplankton community found no clear concentration–response relationship with TPT (Fig. 9). In both types of test system, the 10 μg/l treatment deviated more from the controls than the two highest treatment levels. Monte Carlo permutation testing, however, did not reveal a consistent treatment-related effect at the level of the phytoplankton community (Table 9). Only in the CS cosms were significant differences between treatments found on the first sampling date before (week −1) and after (week 0.4) the TPT treatment.

In the PRC diagrams of both the CS (Fig. 9A) and PS (Fig. 9B) microcosms, the diatom *Cocconeis* shows the highest positive b_k score, while the green alga *Scenedesmus*

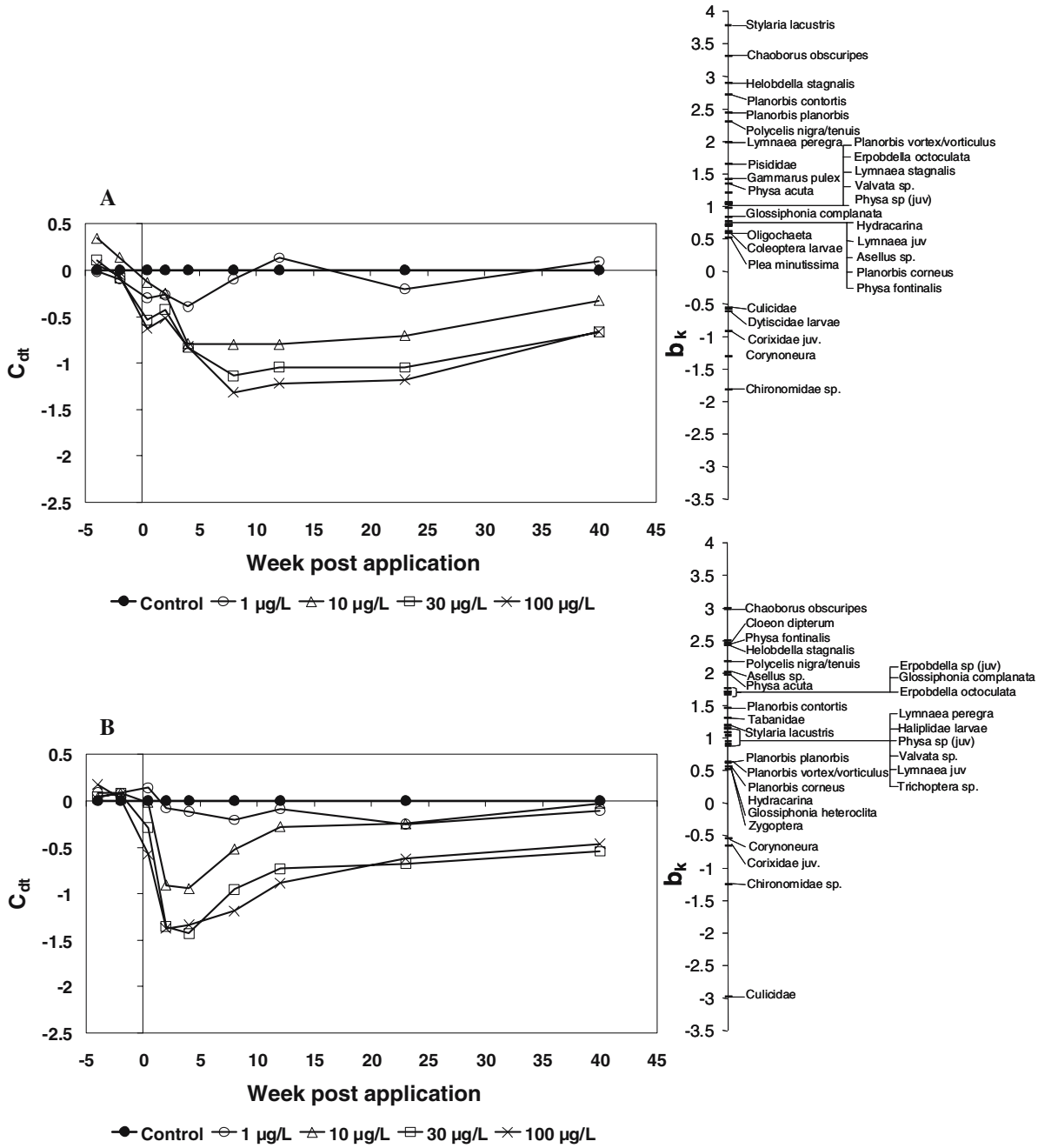

Fig. 4 Principal Response Curves resulting from the analysis of the macroinvertebrate data set from the TPT treated microcosms with clean (**A**) and polluted (**B**) sediment. In the clean sediment cosms, 39% of the variance was explained by treatment and 36% by time. The corresponding percentages for the polluted sediment cosms were 41 and 38. Calculated $NOEC_{community}$ values are presented in Table 6

has the highest negative b_k score. This is in accordance with the observed population dynamics of *Cocconeis* (Fig. 10C, D) and *Scenedesmus* (Fig. 10E, F); these taxa showed an overall decline and an overall increase in densities, respectively, in the 10 µg/l microcosms relative to controls. After TPT application, no clear linear concentration–response relationship was found for *Cocconeis* or *Scenedesmus*. For this reason, the calculated NOEC values of < 1–30 µg/l for *Cocconeis* in the CS systems (Table 4) should be interpreted with caution. This is also true for the NOECs < 1 µg/l calculated for a few other phytoplankton taxa in the CS microcosms, viz. *Phacus* and *Trachelomonas* (Fig. 10G and Table 4). The Williams test assumes a monotonic response, and consequently may show an output that is difficult to interpret when actual responses are non-linear.

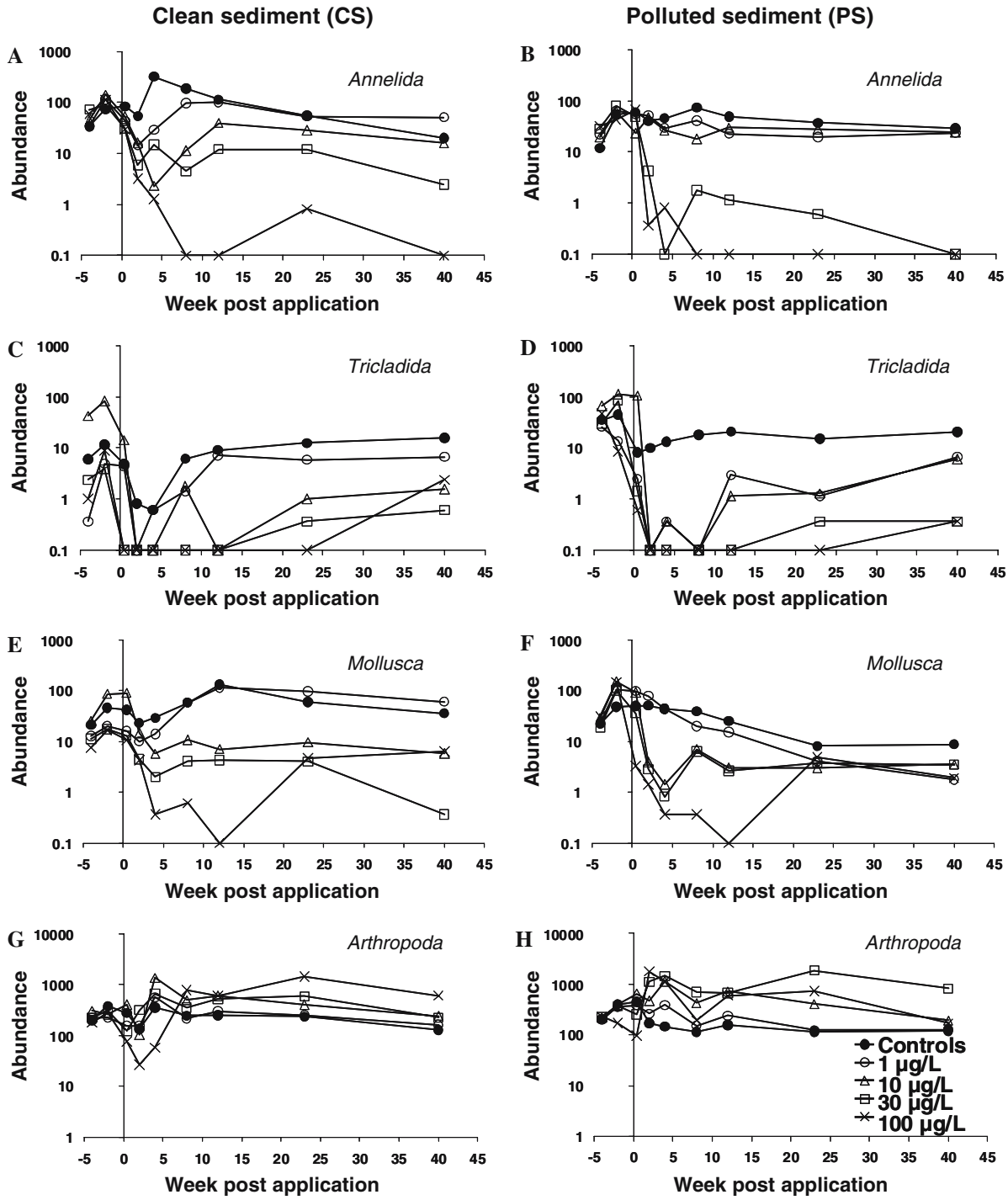

Fig. 5 Dynamics of the macroinvertebrate taxonomic groups Annelida (**A** and **B**), Tricladida (**C** and **D**), Mollusca (**E** and **F**), and Arthropoda (**G** and **H**) in clean sediment and polluted sediment cosms that were treated with TPT. Calculated NOEC values are presented in Tables 4 and 5

In both types of test system, significant treatment-related increases in phytoplankton chlorophyll-*a* were observed, particularly in the PS cosms with the two highest TPT concentrations (Fig. 10A and Table 4; Fig. 10B and Table 5). At the taxon level, this increase could only be confirmed by the response of *Cosmarium*, for which NOEC values of 30 µg/l were calculated in the PS cosms (Fig. 10H, Table 5).

The development of macrophytes in the test systems (as percentage cover) is presented in Fig. 11. The macrophyte community was dominated by *Elodea nuttallii* and developed differently in the two types of microcosm. In the CS

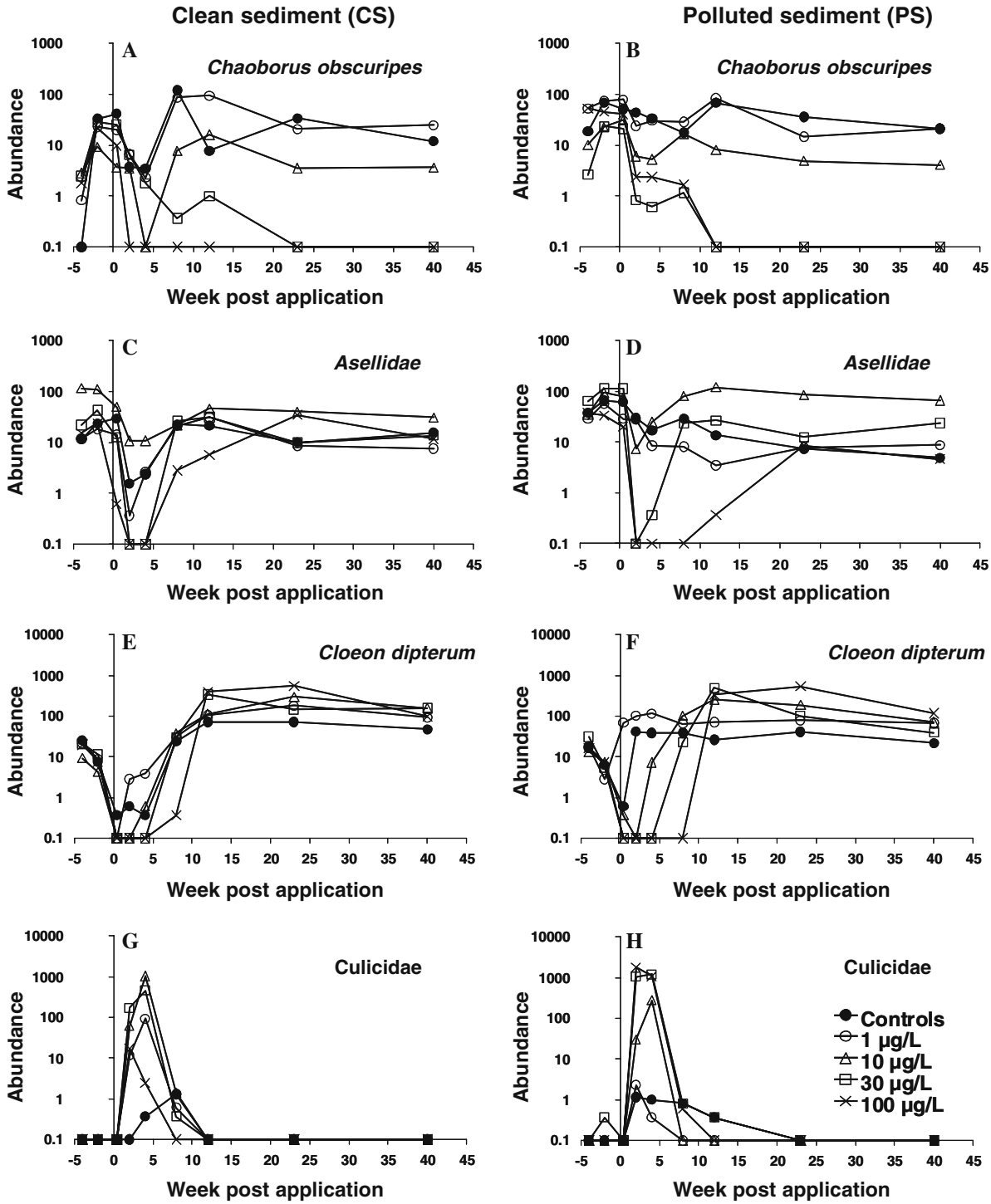

Fig. 6 Dynamics of four arthropod taxa in clean sediment and polluted sediment cosms treated with TPT. The taxa represent a very sensitive species which does not recover (*Chaoborus obscuripes*; **A** and **B**), a sensitive taxon that recovers (*Asellidae*; **C** and **D**), a sensitive taxon that recovers and even increases in abundance (*Cloeon dipterum*; **E** and **F**), and an insensitive taxon that increases in numbers (Culicidae; **G** and **H**). Calculated NOEC values are presented in Tables 4 and 5

cosms, macrophyte growth (Fig. 11A) was initially hindered by high densities of filamentous algae (Fig. 11C). Only after the filamentous algae declined was there an increase in the percentage cover of *Elodea nuttallii*, several weeks after TPT application. In contrast, the PS cosms, in which densities of filamentous algae were low (Fig. 11D),

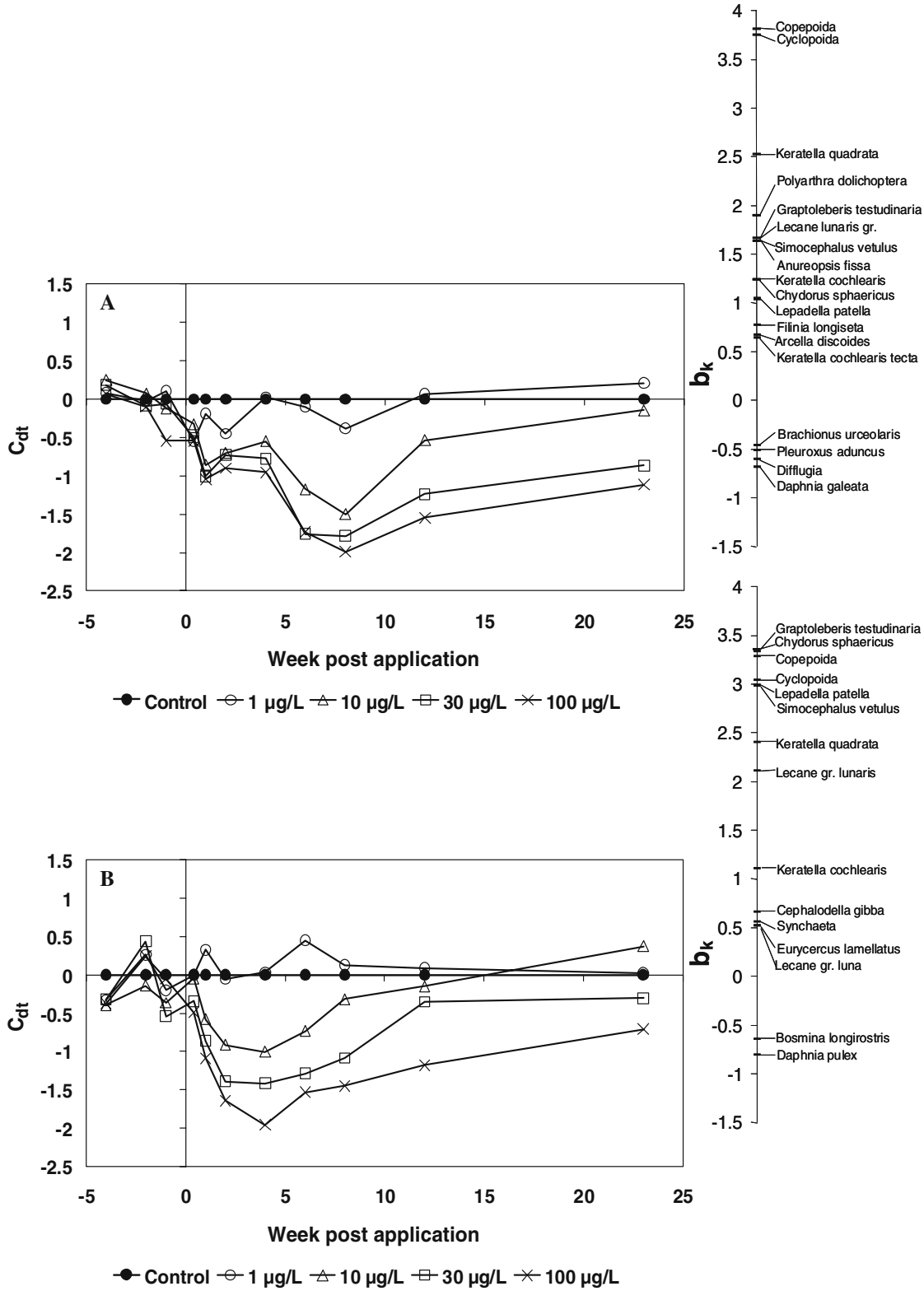

Fig. 7 Principal Response Curves resulting from the analysis of the zooplankton dataset from the TPT treated microcosms with clean (**A**) and polluted (**B**) sediment. In the clean sediment cosms, 38% and 32% of the variance was explained by treatment and time, respectively. The corresponding percentages for the polluted sediment cosms were 46 and 21. Calculated $NOEC_{community}$ values are presented in Table 7

Table 7 Significance of the Monte-Carlo permutation tests and $NOEC_{community}$ values calculated from the PRC analysis of the zooplankton communities in the TPT treated microcosms with clean (CS) and polluted (PS) sediment

Week relative to application	CS cosms		PS cosms	
	P-value	$NOEC_{community}$ (μg/l)	*P*-value	$NOEC_{community}$ (μg/l)
−4	>0.05	>100	>0.05	>100
−2	>0.05	>100	>0.05	>100
−1	>0.05	>100	>0.05	>100
0.4	>0.05	>100	0.031	10
1	0.001	10	0.003	1
2	>0.05	>100	0.001	1
4	0.001	10	0.001	1
6	0.005	10	0.003	1
8	0.002	1	0.004	10
12	0.001	1	0.018	>100
23	0.006	10	0.053	30

showed an increase in the percentage cover of *Elodea* even in the pre-treatment period (Fig. 11B). In the CS cosms, calculated NOEC-values for the percentage cover of *Elodea nuttallii* were 30 μg/l in the period of 2–13 weeks after TPT application (Table 4). In the PS cosms, an NOEC of 10 μg/l was found in weeks 6, 7, 8, and 13, and an NOEC of 30 μg/l in weeks 3, 4, 5, 10, and 12 (Table 5). Macrophyte coverage was most affected at the highest treatment level. In the CS cosms, hardly any *Elodea* developed in the 100 μg/l cosm. In the PS cosms a pronounced decline in *Elodea* was observed 5 weeks after TPT application, without clear signs of recovery up until week 13.

Decomposition

The residual dry weight of the *Populus* leaves in the decomposition assays in the various 2-week incubation

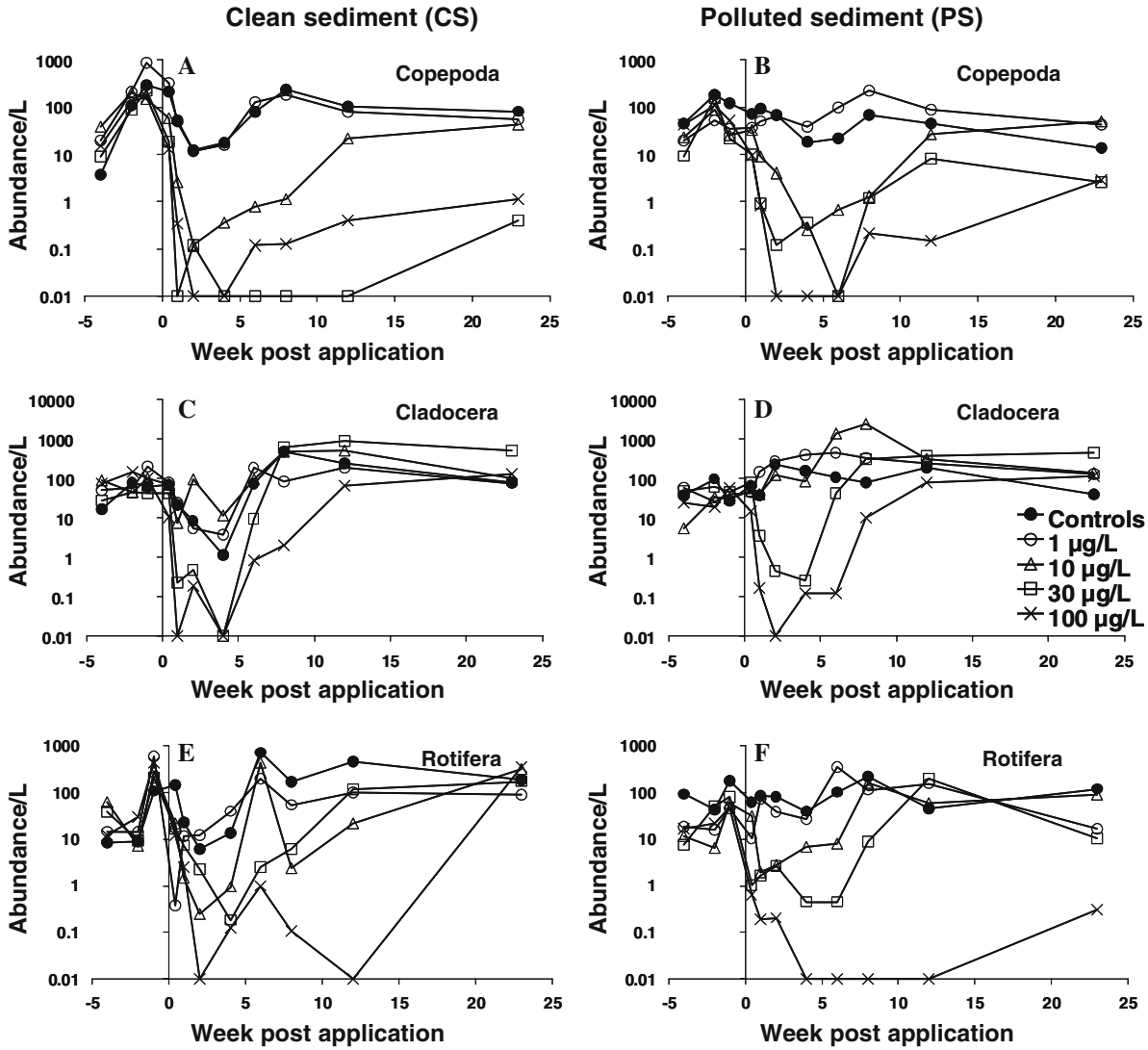

Fig. 8 Dynamics of the main groups of zooplankton, Copepoda (**A** and **B**), Cladocera (**C** and **D**), and Rotifera (**E** and **F**) in clean sediment and polluted sediment cosms treated with TPT. Calculated NOEC values are presented in Tables 4 and 5

Table 8 Maturity Index calculations for the benthic nematode community in the TPT treated microcosms with clean (CS) and polluted (PS) sediment

	Week relative to treatment	Range of Maturity Index over treatments
CS cosms	−3	2.21–2.43*
	2	2.29–2.67*
	12	2.33–2.75*
PS cosms	−3	2.04–3.00*
	2	2.06–2.47*
	12	2.06–2.48*

*No significant treatment-related effects found

periods was approximately 45–55% in both types of test system. No treatment-related effect on litter breakdown was, however, demonstrated in either the CS or the PS cosms.

Discussion

Ecological responses to TPT exposure

In our microcosm experiment, we observed that representatives of several taxonomic groups of freshwater invertebrates showed a clear response to a single application of TPT at treatment levels of 10 μg/l and more (Tables 4 and 5; Figs. 5, 6, 8). Soft-bodied taxa in particular seemed to be most affected. The fact that representatives of Tricladida, Annelida, Mollusca, Crustacea, Insecta and Rotifera all showed a treatment-related decline in abundance suggests that TPT is a compound with a broad biocidal mode of action. These observations in the microcosms are in line with the results of single species toxicity tests with TPT performed in the laboratory (Table 10; for further details see part II, Roessink et al. 2006). Indeed, acute laboratory toxicity data show that several invertebrate groups are sensitive to this compound in the concentration range selected in our microcosm experiment (1–100 μg/l). In our study, the nematode community did not respond to TPT application, nor did individual populations of nematodes. This was unexpected, since several other studies have revealed significant effects of organotin compounds on benthic nematodes (Austen and McEvoy 1997; Fliedner et al. 1997). The reason why the nematode community in our two types of microcosm did not respond to TPT application is probably twofold. First, the most likely exposure route of nematodes to TPT is via the pore water, and the concentrations of TPT measured in the pore water remained relatively low (Fig. 2). Second, the sediments we used originated from floodplain lakes, which are very dynamic systems. The nematode community originating from these systems was, not surprisingly, characterized by colonizers, which are by definition hardy organisms that can withstand dynamic, harsh, and stressful circumstances better than non-colonizing species.

The laboratory data presented in Table 10 indicate that, apart from several invertebrates, algae and vascular plants are also sensitive to TPT application. This, however, is only partly in agreement with our observations in the microcosms. In our study, the macrophyte *Elodea nuttallii* significantly declined at treatment levels of 30 and 100 μg TPT/l (Fig. 11; Tables 4 and 5) whereas its sensitivity in 21-day laboratory single species tests was lower. In the laboratory, an EC_{50} of 11.8 μg/l was recorded for the relative growth of *Elodea nuttallii* (Roessink et al. 2006). Most populations of algae in the microcosms did not show a clear linear treatment-related decline in abundance. This suggests that the extrapolation of results of laboratory single species toxicity tests to predict population-level responses in (experimental) ecosystems may be more difficult for primary producers than for invertebrates. In part, this may be explained by the fact that the measurement endpoint selected for invertebrates in single species toxicity tests is usually mortality and/or immobility, while in algae and macrophytes this is usually growth rate. Growth rate may be more subject to environmental factors that vary in space and time (e.g. light conditions, nutrient concentrations and grazing pressure) than the mortality/immobility endpoint. In addition, populations and communities of algae, which are characterized by an overall short generation time of the organisms, may develop tolerance within a relatively short period of time (Kasai and Hanazato 1995; Blanck and Dahl 1996; Van den Brink et al. 1997; Blanck 2002).

In our microcosm experiment, several populations of algae increased in numbers at lower treatment levels and declined at higher exposure concentrations (see e.g. Fig. 10E, F, G). The non-linear responses observed in these taxa were most probably caused by the interplay between direct and indirect effects. For example, the increase in *Scenedesmus* in the microcosms treated with 10 μg/l relative to controls in the 2–10 weeks after TPT application (Fig. 10E, F) may have been caused by a treatment-related decline in zooplankton densities in the 10 μg/l test systems (Fig. 8), resulting in a reduced grazing pressure on *Scenedesmus*. In contrast, the lower *Scenedesmus* densities relative to the 10 μg/l cosms at the two highest treatment levels might be explained by increased toxicity of TPT. This was further substantiated by single species tests performed with *Scenedesmus quadricauda*. In this species, calculated EC50 values at 48, 72, and 96 h after TPT application were 352.9, 29.1, and 36.0 μg/l, respectively (Roessink et al. 2006), suggesting that at even longer periods after TPT application, 10 μg/l could cause toxic

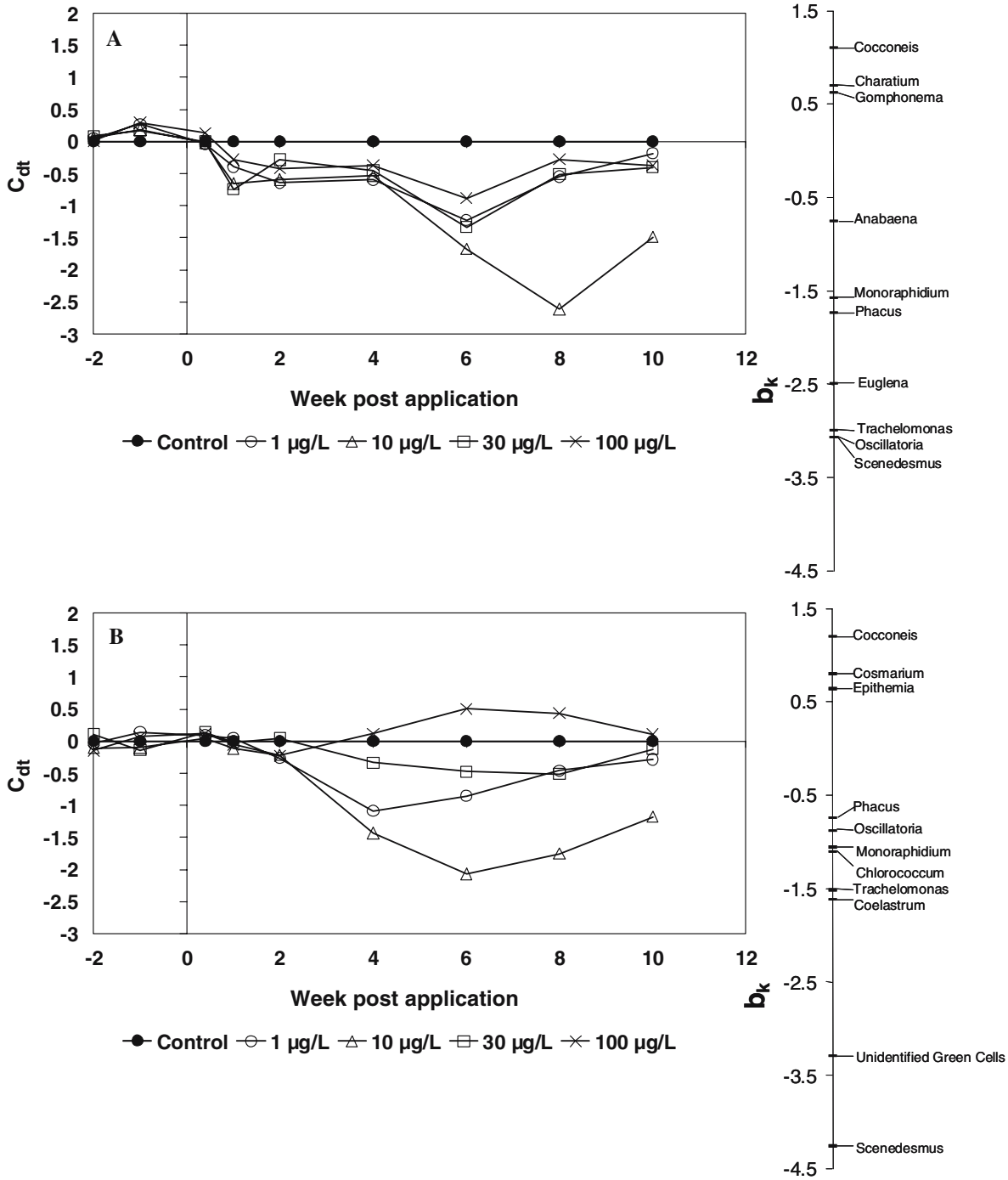

Fig. 9 Principal Response Curves resulting from the analysis of the phytoplankton data set from the TPT treated microcosms with clean (**A**) and polluted (**B**) sediment. In the clean sediment cosms, 33% of the variance was explained by treatment and 38% by time. The corresponding percentages for the polluted sediment cosms were 38 and 28. Calculated $NOEC_{community}$ values are presented in Table 9

effects which overrule the positive effect of the release from grazing pressure. Similar response patterns have been found for daphnids in insecticide-treated microcosms which were also inhabited by the more sensitive predator *Chaoborus obscuripes* (Roessink et al. 2005). The temporary increase in phytoplankton chlorophyll-*a* levels at the two highest treatment levels (Fig. 10A, B) might also be regarded as an indirect effect due to the reduced grazing by zooplankton. At the population level, however, this temporary increase in phytoplankton biomass could only be confirmed for a few phytoplankton taxa (e.g. *Cosmarium*, Fig. 10H). The increase in phytoplankton chlorophyll-*a*

Table 9 Significance of the Monte-Carlo permutation tests and $NOEC_{community}$ values calculated from the PRC analysis of the phytoplankton communities in the TPT treated microcosms with clean (CS) and polluted (PS) sediment

Week relative to application	*P*-value	
	CS cosms	PS osms
−2	>0.05	>0.05
−1	0.03	>0.05
0.4	0.03	>0.05
1	>0.05	>0.05
2	>0.05	>0.05
4	>0.05	>0.05
6	>0.05	>0.05
8	>0.05	>0.05
10	>0.05	>0.05

levels that we observed in our microcosms is in accordance with the results obtained by Fliedner et al. (1997) and Jak et al. (1998), who performed model ecosystem experiments with the organotin compounds azocyclotin and TBT, respectively. In their experiments, phytoplankton was inhibited at higher concentrations than the zooplankton, and direct toxic effects on zooplankton enabled the phytoplankton to increase at certain treatment levels.

In microcosm and mesocosm experiments, a clear distinction between direct and indirect effects can only be made on the basis of additional information. This information includes laboratory toxicity data on taxa that show a treatment-related response in the experimental ecosystems, as well as ecological information on the position of the affected organisms in the aquatic food web (see e.g. Baird et al. 2001; Fleeger et al. 2003; Traas et al. 2004). Treatment-related increases in the abundance of populations are most likely the result of indirect effects on relatively tolerant species. Besides the indirect effects on phytoplankton, we observed temporary increases in the abundance of insect taxa in particular, viz., Culicidae (Table 5, Fig. 6G, H), Chironomidae (Tables 4 and 5), *Cloeon dipterum* (Tables 4 and 5; Fig. 6E, F), Corixidae (Table 5), and Dytiscidae larvae (Table 4). Literature reports show that representatives of Culicidae (Hynes 1963) and Chironomidae (De Haas et al. 2002) are relatively tolerant to pollutants. The period of increase in Culicidae (weeks 2–4) coincided with a decrease in dissolved oxygen concentration. The air-breathing larvae of Culicidae are well-adapted to low oxygen levels in the water and feed on all types of dead and living, fine-particulate organic matter (Tun-Lin et al. 2000; Koenraadt and Takken 2003). During the first weeks after treatment, food in the form of fine particulate organic matter was abundant, partly as a result of the toxic effects of TPT on plants and animals, and partly because of the increase in microorganisms due to the use of ethanol as a solvent for TPT. The other insect taxa mentioned above showed treatment-related increases in abundance much later, at least 8 weeks after TPT application. Given the fact that many other populations of water organisms were negatively affected for a long time, it is difficult to explain with certainty which food web interactions played a major role in the increase in insect taxa. A possible explanation is a decreased competition for food due to a decline of other populations that utilize the same food source. For example, the increase in numbers of the periphyton grazer *Cloeon dipterum* at the highest treatment level (after its initial decline) may have been caused by the long-term decline in Mollusca and *Stylaria lacustris*, which also feed on periphyton. Another explanation might be the release from predation due to a treatment-related decline in carnivores such as Tricladida and Hirudinea.

Comparing our results with those of other studies is difficult, because the only published freshwater microcosm study with an organotin compound we are aware of is that performed with the pesticide azocyclotin (Fliedner et al. 1997). Most other studies have been performed in the marine environment with TBT, focusing on responses by plankton, periphyton, and benthic meiofauna (Dahl and Black 1996; Austen and McEvoy 1997; Jak et al. 1998; Evans et al. 2000; Petersen and Gustavson 2000). Furthermore, the published information on the ecological impact of fungicides in freshwater ecosystems is generally also very limited. The responses that we observed in our experimental freshwater ecosystems due to TPT application are more or less comparable with the effects observed in studies of macrophyte-dominated freshwater microcosms treated with the fungicide carbendazim (Cuppen et al. 2000; Van den Brink et al. 2000). These studies also found that representatives of Tricladida, Oligochaeta, Hirudinea, Mollusca, Rotifera, and Crustacea were negatively impacted, again indicating that certain fungicides may have a broad biocidal mode of action in freshwater ecosystems.

Influence of sediment quality

The two types of microcosm used in our experiment were stocked with relatively clean or polluted sediments collected in floodplain lakes of the river Waal (in the Rhine catchment). The difference in sediment quality between these test systems related mainly to PAHs, PCBs, metals, organic matter, and nutrients (Table 1). These differences in sediment quality affected the structure of the aquatic communities that developed in the microcosms. In the microcosms with 'polluted' sediments, a dense vegetation of the macrophyte *Elodea nuttallii* had already developed at the start of the experiment, when TPT was applied. In contrast, the development of *Elodea nuttallii* in the microcosms with 'clean' sediments was retarded, partly

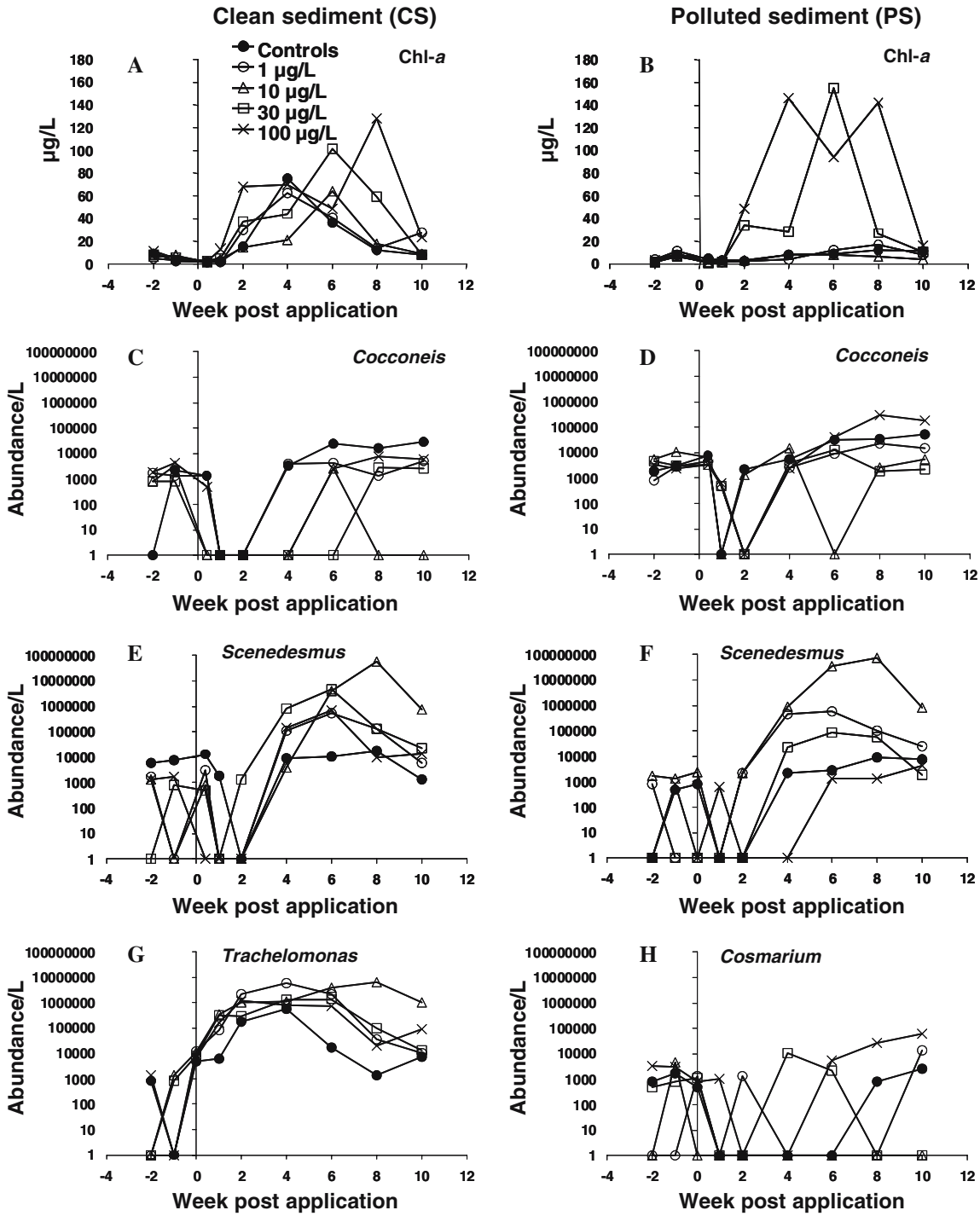

Fig. 10 Dynamics of phytoplankton Chl-*a* (**A** and **B**) and of the four taxa found to be most important in the PRC analysis (*Cocconeis*; **C** and **D**, *Scenedesmus*; **E** and **F**, *Trachelomonas*; G, and *Cosmarium*; **H**) in TPT treated microcosm with clean sediment and polluted sediment. Calculated NOEC values are presented in Tables 4 and 5

because of lower nutrient levels and partly by a bloom of filamentous algae (Fig. 11). These differences in macrophyte growth were most probably driven by differences in nutrient content of the sediments used. Differences in the dominance of phytoplankton and invertebrate populations between microcosm types may have been caused directly by differences in sediment quality, or indirectly by differences in macrophyte biomass. Several field studies have revealed that both sediment-bound toxicants (Peeters et al. 1999; Reinhold-Dudok van Heel and Den Besten 1999;

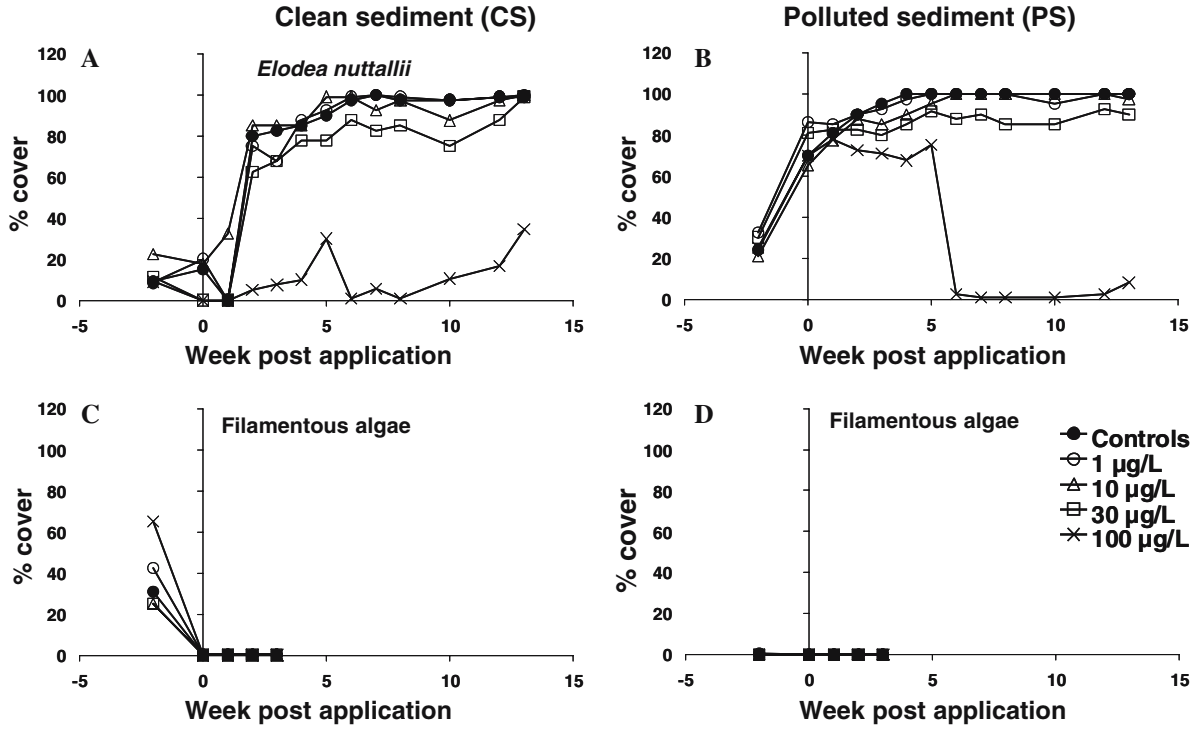

Fig. 11 Dynamics in % cover of the vascular plant *Elodea nuttallii* (**A** and **B**) and of filamentous algae (**C** and **D**) in TPT treated microcosms with clean (CS) and polluted (PS) sediments. Calculated NOEC values are presented in Tables 4 and 5

Table 10 Acute EC_{50}/LC_{50} toxicity data of TPT for several taxonomic groups of aquatic organisms available from the literature

Taxa	Range EC_{50}/LC_{50} (µg/l)	References
Algae (72 h)	1–28	Fargasová (1997) and Roessink et al. (2006)
Vascular plants (21 days)	5–199	Roessink et al. (2006)
Tricladida (96 h)	6–7	Roessink et al. (2006)
Annelida (96 h)	2–17	Fargasová (1997)
Mollusca (96 h)	7–12	Roessink et al. (2006)
Microcrustacea (48 h/96 h)	1–16	Roessink et al. (2006) and De Zwart (2002)
Macrocrustacea (96 h)	9–96	Roessink et al. (2006)
Insecta (96 h)	0.33–205	Fargasová (1997) and Roessink et al. (2006)

Peeters et al. 2001) and beds of aquatic vascular plants (LaLonde and Downing 1992; Brock and Van der Velde 1996; Scheffer 1998) have a significant effect on invertebrate communities.

That the toxicants in the sediment of the PS microcosms pose risks to benthic organisms is suggested by the sediment quality criteria that are currently used in the Netherlands (Crommetuijn et al. 2000; Van Griethuysen et al. 2003). When concentrations of individual compounds remain below the maximum permissible concentration (MPC), the ecotoxicological hazard of the compound is considered to be tolerable. In the clean sediments that we used to construct the CS microcosms, standardized concentrations (25% lutum, 10% organic matter) of individual metals, PAHs, and PCBs were all well below MPC values (De Haas 2004). In the sediment of the PS microcosms, however, the standardized concentrations of several PAHs (anthracene, phenantrene, benzanthracene) and PCBs (PCB052, PCB101, PCB118, PCB138, PCB153, PCB180) were a factor 2–5 above MPC values, while several other compounds (including the metals Cu and Zn) were only slightly below their MPC values (De Haas 2004). That the sediment of the PS microcosms was toxic to certain benthic organisms is further substantiated by the findings of 10-day whole sediment bioassays, which showed that survival of larvae of the mayfly *Ephoron virgo* was approximately 48% in tests using sediment from the controls of the PS cosms, against 78% in sediments from the controls of CS microcosms (De Haas et al. 2005). The response of this sensitive mayfly suggests that the total cocktail of background pollutants in the sediment of the PS microcosms may have affected sensitive benthic invertebrates.

The most important aim of our microcosm experiment was not to study differences in the development of the aquatic community in microcosms differing in sediment

quality. The question at stake was whether sediment-bound pollutants affect the response (in terms of sensitivity and resilience) of aquatic populations and communities to additional stressors. In our experiment, this additional stressor was triphenyltin acetate (TPT), applied in a concentration range (0–100 μg/l). Furthermore, the application of the carrier solvent ethanol can also be regarded as an additional stressor in our experiment. The carrier solvent, however, was applied to all microcosms, including the controls. In both types of test system, ethanol caused a temporary drop in dissolved oxygen levels. This drop was more pronounced in the CS cosms, since DO levels had already sharply declined in the pre-treatment period due to the senescence of the filamentous algae bloom (Fig. 3). During the first weeks after TPT application, this more pronounced drop in DO levels in the CS cosms may have obscured some direct effects of TPT. This explains why in the PRC curves for macroinvertebrates (Fig. 4), zooplankton (Fig. 7) and phytoplankton (Fig. 9), the responses were less pronounced in the CS cosms than in the PS microcosms during the first 4 weeks after TPT application. Nevertheless, in both types of test system, the single application of TPT had a long-term impact on several aquatic organisms, so that pronounced treatment-related responses were still observed when DO oxygen levels had returned to normal levels.

Following the procedures described in Van Wijngaarden et al. (2005) and Anderson and Ter Braak (2003), Monte-Carlo permutation tests can also be used to test whether communities differ significantly between types of test system, and whether there are interactions between the factors "treatment" and "system type". Results of such an analysis (Table 11) fully support the above claims that the structure of the invertebrate communities (in particular the macroinvertebrate community) differed significantly between the two types of test system and that interaction between community structure and TPT treatment regime only occurred during the first weeks after TPT application, when DO levels were much lower in the CS microcosms.

In our paper we expressed the responses of the aquatic populations and communities in terms of nominal treatment levels (= initial peak concentrations). This may complicate the extrapolation of out microcosm results to the field, since peak concentrations are often not monitored in the field. In addition, the long-term response that we observed in our test systems suggests chronic toxicity. To facilitate the extrapolation of our data to measured data from the field initial nominal concentrations used in the present study have been translated in Tables 12 and 13 into chronic twenty-one day exposure data. As a reference point concentrations at twenty-one days have been selected because the present study focusses on invertebrates and the chronic toxicity test for the standard invertebrate test species (*Daphnia magna*) last for twenty-one days. Also Fig. 2 shows that concentrations in sediment and pore-water remain rather constant indicating that in our study concentrations at 21 days have sufficient predictive value for longer term exposure concentrations in pore-water and sediment. In Tables 12 and 13 exposure via the water column is expressed in three ways: initial nominal concentrations, concentration at 21 days, and the time-weighted average (TWA) over a twenty-one day period.

These summary tables use five effect classes (adapted after Brock et al. 2000) to facilitate the comparison of TPT-related responses between the two types of microcosm. TPT-related effects were observed in both CS cosms and PS cosms, at all treatment levels. At the lowest treatment level of 1 μg/l, however, effects on sensitive community endpoints in both types of test system were slight and transient (effect class 2), while observed effects on sensitive population endpoints were followed by full recovery (effect classes 3 and 4 in CS cosms and PS cosms,

Table 11 Results of Monte-Carlo permutation tests (*P*-values) on the combined macro-invertebrate and combined zooplankton datasets of the microcosms constructed with clean (CS) and polluted (PS) sediments and treated with TPT

Week	Macroinvertebrates Treatment	System type	Interaction	Zooplankton Treatment	System type	Interaction
−4	>0.05	0.005	>0.05	>0.05	>0.05	>0.05
−2	>0.05	0.035	>0.05	>0.05	>0.05	>0.05
−1	-	-	-	>0.05	>0.05	>0.05
0.4	0.025	0.040	>0.05	>0.05	>0.05	>0.05
1	-	-	-	0.002	0.015	>0.05
2	0.005	0.005	0.010	>0.05	0.005	0.003
4	0.005	0.005	0.025	0.004	0.005	0.002
6	-	-	-	0.001	0.035	>0.05
8	0.005	0.015	>0.05	0.001	0.015	>0.05
12	0.005	0.005	>0.05	0.001	>0.05	>0.05
23	0.005	0.040	>0.05	0.001	>0.05	>0.05
40	0.005	>0.05	>0.05	-	-	-

The statistical significance was tested of the treatment, system type, and interaction between "treatment" and "system type" following the procedures described in Van Wijngaarden et al. (2005) and Anderson and Ter Braak (2002)

Table 12 Summary of effects observed in the clean sediment cosms treated with the fungicide TPT

CS-cosms				
Initial nominal concentrations	1 µg/l	10 µg/l	30 µg/l	100 µg/l
Concentration 21-days	0.1 µg/l*	0.7 µg/l	2.1 µg/l	7.0 µg/l*
TWA 21-days	0.3 µg/l*	3.1 µg/l	9.2 µg/l	30.8 µg/l*
Pore water concentration 21-days	6.2 ng/l*	62.2 ng/l	186.7 ng/l	118.5 ng/l*
Sediment concentration 21-days	6.8 µg/kg*	68.1 µg/kg	204.4 µg/kg	681.2 µg/kg*
Community-level responses				
PRC macroinvertebrate	2	4	5	5
PRC zooplankton	1	4	5	5
PRC benthic nematodes	1	1	1	1
PRC phytoplankton	1	2	2	2
Plankton Chl-*a*	1	1	2	3
Water quality parameters	1	2	3	3
Decomposition	1	1	1	1
Population-level responses				
Tricladida	1	4–5	4–5	4–5
Annelida	3	3–4	5	5
Mollusca	2	5	5	5
Insecta	1	3–4	5	5
Macrocrustacea	1	1	2	3
Microcrustacea	2	3–4	5	5
Rotifera	3	3	4	4
Benthic nematoda	1	1	1	1
Phytoplankton	2–3	2–3	5	5
Macrophytes	1	1	1	5

The numbers in the table refer to effect classes adapted after Brock et al. (2000). 1: No effects, 2: Slight transient effects, 3: Clear short-term effects, recovery time < 8 weeks, 4: Clear medium-term effects, recovery time > 8 weeks but within the period studied, 5: Clear long-term effects, full recovery not observed. TWA= Time Weighted Average, * extrapolated from calculated concentrations in the 10 and 30 µg/l treatments

Table 13 Summary of effects observed in the polluted sediment cosms treated with the fungicide TPT

PS-cosms				
Initial nominal concentrations	1 µg/l	10 µg/l	30 µg/l	100 µg/l
Concentration 21-days	0.1 µg/l *	0.9 µg/l	2.8 µg/l	9.2 µg/l *
TWA 21-days	0.4 µg/l *	3.8 µg/l	11.5 µg/l	38.2 µg/l *
Pore water concentration 21-days	3.9 ng/l *	39.5 ng/l	118.5 ng/l	394.9 ng/l *
Sediment concentration 21-days	4.6 µg/kg*	46.1 µg/kg	138.2 µg/kg	460.6 µg/kg*
Community-level responses				
PRC macroinvertebrate	2	4	5	5
PRC zooplankton	1	3	3–4	5
PRC benthic nematodes	1	1	1	1
PRC phytoplankton	1	1	1	1
Plankton Chl-a	2	3	3–4	4
Water quality parameters	1	3	4	4
Decomposition	1	1	1	1
Population level responses				
Tricladida	3–4	3–4	5	5
Annelida	4	5	5	5
Mollusca	2	4	4	4
Insecta	2	5	5	5
Macrocrustacea	1	1	3	4
Microcrustacea	1	3	4	4–5
Rotifera	2	3	3–4	5
Benthic nematoda	1	1	1	1
Phytoplankton	1	1	1	4
Macrophytes	1	1	3–4	5

The numbers in the table refer to effect classes described in detail in Brock et al. (2000). 1: No effects, 2: Slight transient effects, 3: Clear short-term effects, recovery time < 8 weeks, 4: Clear medium-term effects, recovery time>8 weeks, 5: Clear long-term effects, full recovery not observed. TWA=Time Weighted Average, *extrapolated from calculated concentrations in the 10 and 30 µg/l treatments

respectively). At the two highest treatment levels (30 and 100 μg TPT/l), class 5 effects (clear effects without full recovery) were recorded in both the CS and PS cosms, and for both community-level and population-level endpoints.

In agricultural area's water concentrations of 4.3 ng/l (Stäb 1995) and in marinas concentrations up to 90 ng/l have been reported (Fent and Hunn 1995). In the present study water concentrations are still higher on day 21 after application, although waterconcentrations in the 1 μg/l treatment do approximate water concentrations in marinas (Tables 12 and 13). In freshwater sediments, TPT concentrations up to 70 and 380 μg/kg have been reported for, respectively, Lake Westeinder (The Netherlands) and Lake Lucerne (Swiss) (Stäb 1995; Fent and Hunn 1995) which is comparable with sediment concentrations found in the 30 μg/l treatment of the present study (Tables 12 and 13).

Between the two types of system tested in the present study we found a few differences in the intensity and/or duration of TPT-related responses (Tables 12 and 13). In the CS cosms, more pronounced responses occurred in the zooplankton community (PRC zooplankton) in that no recovery was observed at the 30 μg/l treatment level, while full recovery was observed at this level in the PS cosms. A few phytoplankton populations showed a more sensitive response at the three lowest treatment levels in the CS cosms than in the PS cosms. In contrast, phytoplankton chlorophyll-*a* showed a more pronounced increase in the systems treated with 10 and 30 μg/l in the PS cosms than in the CS cosms. Furthermore, at least one sensitive endpoint for Tricladida (at 1 μg/l), Annelida, and Insecta (at 10 μg/l) and for macrophytes (at 30 μg/l) appeared to respond more sensitively in the PS cosms. Overall, however, it can be concluded that the responses of the most sensitive endpoints for each category listed in Tables 12 and 13 hardly differed between the two types of test system. This is in agreement with the very similar TPT concentration dynamics in the overlying water of the CS and PS cosms during the first weeks after application (Fig. 1).

Nevertheless, focusing on long-term community responses, the PRC curves of the macroinvertebrate and zooplankton communities indicate that the overall rate of recovery was somewhat faster in the PS cosms than in the CS systems (Figs. 4, 7; Tables 6 and 7). A possible explanation for this might be the higher organic matter content of the sediments in the PS cosms, as well as the larger macrophyte biomass in these test systems at the time of TPT application. Due to its high affinity with organic matter and its resistance to breakdown (Looser et al. 2000), TPT is expected to accumulate in the macrophyte and sediment compartments. Indeed, our microcosm study showed that TPT appeared to be very persistent in the sediments, but total concentrations in sediments of the PS cosms were somewhat lower than in the CS cosms (Fig. 2), which is explained by the greater biomass of macrophytes in the PS systems at the time of TPT application. Since long-term effects were observed after a single application of TPT, it is plausible that at least part of the sorbed fraction of TPT remained bioavailable. There is a relatively high potential for bioaccumulation of TPT in freshwater organisms, suggesting that uptake by ingestion of particle-bound TPT should not be ignored (Fent 1996; Looser et al. 2000). Sequestering of TPT in organic material which is processed rapidly by benthic biota and degradation of macrophyte-associated TPT, however, may have been stronger and faster in the PS cosms, which were characterized by a higher organic matter content of the sediment and a greater initial macrophyte biomass. This may have decreased the long-term bioavailability of sorbed TPT at a somewhat faster rate in the PS systems than in the CS microcosms. This may have masked possible effects of background pollutants because the overall response would be the sum of several stress factors. A more rapidly declining TPT effect plus a background pollutant effect can be of the same magnitude as the TPT effect in the CS cosms, thus creating a similar overall response.

The observation that overall responses of sensitive populations were rather similar between test system types is further substantiated by the fact that EC_{50} values could be calculated for several invertebrate populations sampled in the microcosms, resulting in very similar species sensitivity distribution (SSD) curves between the CS and PS microcosms (for detailed results see part II; Roessink et al. 2006). Hazardous concentrations for 5% of the species (HC5) calculated on the basis of these microcosm–SSD curves in sampling weeks 2–8 after TPT treatment ranged from 0.3 to 0.6 μg/l for the CS cosms and from 0.2 to 0.6 μg/l for the PS cosms. These data indicate that threshold concentrations of TPT for effects on invertebrate populations, calculated by means of SSD, were also very similar for the microcosms with clean and polluted sediments. Basing SSD curves not on initial nominal concentrations but on concentrations 21 days post-application which is approximately a tenfold lower results in a similarly decreased HC5 value ranging from 20 to 60 ng/l which is lower than measured in freshwater marinas (Fent and Hunn 1995).

In conclusion, sediment quality impacted on the structure and functioning of the aquatic community, but no effects of background pollutants were detected on the response of the aquatic community to TPT. Threshold concentrations and measurement endpoints indicative of direct toxic effects of TPT were very similar between the microcosms with clean and those with polluted sediments. Long-term recovery of the macroinvertebrate and zooplankton communities tended to be somewhat faster in the

polluted sediment systems, possibly because of a slightly stronger sequestering of TPT sorbed to organic matter and the higher productivity of primary producers in these systems.

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