Impact of Sediment-Spiked Fludioxonil On Benthic Macroinvertebrates And Zooplankton From The Yangtze River Delta, China

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Abstract

In China, the fungicide fludioxonil, that accumulates and persists in sediments, has a widespread agricultural use to control various fungal diseases. Its residues may cause toxic effects to benthic and pelagic aquatic fauna, thereby impacting ecosystem service functions of aquatic ecosystems.

To assess the environmental risks of fludioxonil, sediment-spiked single-species toxicity tests with benthic macroinvertebrates as well as a sediment-spiked indoor freshwater microcosm experiment were performed. The microcosm test systems were populated with benthic invertebrates also used in the single-species tests. In all experiments artificial sediment was used.

The single-species tests were conducted with 8 benthic macroinvertebrates covering different taxonomic groups typical for the Yangtze River Delta, China. The 28d-EC_{10} values thus obtained were used to construct species sensitivity distributions (SSDs) and the hazardous concentration to 5% of the species tested (HC_5) was used to derive a provisional long-term Tier-2 regulatory acceptable concentration (RAC) for benthic invertebrates by applying an assessment factor of 3.

The microcosm experiment was conducted to study treatment-related responses of benthic macroinvertebrates and pelagic zooplankton. The lowest No Observed Effect Concentrations (NOECs) of benthic invertebrate and of pelagic zooplankton populations were used to derive provisional long-term Tier-3 RACs for, respectively, benthic and pelagic invertebrates by applying an assessment factor of 2. The RACs thus obtained were compared with Predicted Environmental Concentrations (PECs) of fludioxonil in overlying water and the sediment compartment of edge-of-field ponds after application of this fungicide in rice, grape, wheat, maize and tomato crops. Overall, the risks of long-term exposure of fludioxonil to benthic invertebrates and pelagic zooplankton species in these Chinese edge-of-field ponds appear to be relatively small, but some risks cannot be excluded for ponds adjacent to rice.

Highlights

- There is a lack of information on exposure and toxicity of sediment-spiked fludioxonil on benthic macroinvertebrates and zooplankton from the Yangtze River Delta, China.
- Sediment-spiked single-species toxicity tests and indoor freshwater microcosm experiment were conducted with benthic macroinvertebrates and pelagic zooplankton covering different taxonomic groups typical for the Yangtze River Delta, China.
- The clam *Corbicula fluminea* was the most sensitive benthic macroinvertebrate. The *Daphnia carinata*, *Moina macrocopa* and *Dolerocypris sinensis* were the sensitive zooplankton populations. .
- The potential risks on zooplankton could not be identified when fludioxonil was used in rice, grape, wheat, maize and tomato crops but for benthic macroinvertebrates a risk quotient (RQ) larger than 1 was calculated when the fungicide was used in rice.

Introduction

Sediments may act as sink and exposure route of lipophilic pesticides, potentially giving rise to adverse ecological effects on benthic populations and communities (Warrant et al. 2003; Nowell et al. 2016; Boyle et al. 2016; Brock, et al. 2016; McKnight et al. 2015). To assess benthic organisms from potential exposure to pesticides that accumulate in sediments, prospective effect assessment procedures for sediment organisms have been proposed (EFSA 2015; Diepens et al. 2017). Nevertheless, information on effects of sediment-exposure of pesticides on benthic organisms others than standard test species is relatively scarce (Diepens et al. 2014; EFSA 2015; Yin et al. 2018; Brock et al. 2020). In China, an ERA approach for sediment-dwelling organisms is not yet implemented in the authorization procedure of pesticides if they are predicted to accumulate in the sediment compartment. Consequently, results of sediment-spiked toxicity tests with pesticides and benthic invertebrates are not often published in Chinese regulatory reports until now.

Compared to insecticides and herbicides, the potential environmental risks of fungicides received less attention in the open literature (Köhler and Triebskorn 2013; Wightwick et al. 2012; Zubrod et al. 2019), although fungicides may enter surface waters by spray drift, surface run-off and drainage (Komarek et al. 2010; Kookana et al. 1998; Wightwick et al. 2012). Particularly if the K_{ow} and lipophilicity of the fungicide is high, risks to sediment-dwelling organisms cannot be excluded (EFSA 2015). In addition, many fungicides may have biocidal properties, affecting different taxonomic groups and ecological processes in aquatic ecosystems (Maltby et al. 2009; Rico et al. 2019).

Fludioxonil was selected as benchmark compound in this study, since in China this fungicide is extensively used for reducing the disease caused by various fungi in crops like grapes, rice and wheat (Hui 2016). In addition, recent experimental experience on environmental fate and ecotoxicological effects of fludioxonil in the Netherlands could be used to design the Chinese experiments presented in this paper (Yin et al. 2018; Brock et al. 2020). Furthermore, this also offered the possibility for a geographical comparison of the sensitivity of benthic and pelagic species and communities between the Netherlands and China.

Fludioxonil is a non-systemic and broad-spectrum fungicide. It strongly interferes with mycelia growth and stimulates glycerol synthesis via the mitogen-activated protein kinase pathway(Kanetis et al. 2008, Duan et al. 2013). Fludioxonil is stable to hydrolysis but may rapidly photodegrade in water. It has a low solubility in water (1.8 mg/L) and a high mean partitioning coefficient between soil organic carbon and water (Koc = 145,600 mL/g OC) (EFSA 2007), implying that, when present in surface water, fludioxonil will rapidly adsorb to sediment from the water phase. It was found that fludioxonil has a long persistence in sediments of edge-of-field surface waters since residues of this fungicide were present up to 2 years after its use in grape vineyards (Bermúdez-Couso et al. 2007). Fludioxonil is persistent in aerobic aquatic water-sediment systems, with half-lives of 737 and 643 days in pond and river systems (Health Canada 2016). Several publications showed that fludioxonil has biocidal properties and that in aquatic ecosystems several taxonomic groups, including sediment-dwelling organisms, may be relatively sensitive (Brock et al. 2020; Höss et al. 2019; Yin et al. 2018).

In China, the application rate of fludioxonil reported for rice crops is 0.026 a.s. kg/ha while that is 0.126 a.s. kg/ha for grapes, 0.0088 for wheat and maize and 0.15 kg a.s./ha for tomato (see http://www.chinapesticide.org.cn/). Moreover, in China the fungicide fludioxonil was identified as emerging chemical of concern because of its high or increasing use rates and ferquent detection in surface waters (Zhang et al. 2015; Zhang et al. 2017). For this reason, the studies presented in this paper focused the aquatic risks of fludioxonil, with reference to responses of benthic macroinvertebrates and zooplankton in sediment-spiked test systems.

The purpose of this paper is fourfold; (i) to generate chronic toxicity values for benthic invertebrates typical for the Yangtze River Delta in sediment-spiked single species toxicity tests, (ii) to study the fate of sediment-spiked fludioxonil and population- and community-levels responses of benthic invertebrates and zooplankton from the Yangtze River Delta in laboratory microcosms, (iii) compare the treatment-related responses observed in our test systems with those of similar experiments conducted in the Netherlands, and (iv) to evaluate possible environmental risks of fludioxonil application in the crops grapes, rice, wheat, maize and tomato for sediment-dwelling macroinvertebrates and zooplankton in Chinese edge-of-field surface ponds.

Materials And Methods

2.1 Test species in single species toxicity tests

8 different benthic species belonging to seven different taxonomic orders were selected to conduct sediment-spiked single species toxicity tests with fludioxonil. We selected two species of Oligochaeta worms, *Branchiura sowerbyi* and *Limnodrilus hoffmeisteri* both belonging to Tubificidae, one bivalve, *Corbicula fluminea* (Veneroida), one snail, *Physa acuta* (Basommatophora), one turbellarian worm, *Dugesia japonica* (Tricladida), one leech, *Hirudo nipponia* (Arhynchopdellida), one insect, *Dicrotendipes pelochloris* (Diptera), and one shrimp *Neocaridina denticulata* (Decapoda). Information about origin, size/life-stage and test conditions of these 8 benthic species is presented in Table 1.

Table 1
Species name, origin, life-stage and testing conditions for selected benthic species in the 28-d sediment-spiked toxicity tests with fludioxonil using artificial sediment.

Taxon	Origin test species	A: Mean length in mm B:Mean wet weight in mg	No of replicate test systems to study treatment related effects	Individuals per test vessel(% mortality in controls at day28)	Treatment: Geometric mean test concentrations A: mg a.s./kg dry weight sediment B: µg a.s./L pore water	DO(mg/L) ± SD PH ± SD Temp(°C) ± SD
Branchiura sowerbyi (Oligochaeta)	Outdoor ditches in Machege	A: not measured B: 52.4 ± 2.1	Controls(n = 5) Treatments(n = 5)	10 (0%)	A:0-2.48-4.78-9-18.69-36.45 B:0-23.65-45.41-85.36-176.92-345.16	DO = 8.54 ± 0.07 PH = 7.42 ± 0.04 T = 19.88 ± 0.27
Limnodrilus hoffmeisteri (Oligochaeta)	Outdoor ditches in Machege	A: not measured B: 3.74 ± 0.47	Controls(n = 5) Treatments(n = 5)	10 (0%)	A:0-5.4-10.42-20.44-40.11-80.54 B:0-49.58-95.44-187.12-370.29-736.66	DO = 8.53 ± 0.01 PH = 7.41 ± 0.04 T = 19.9 ± 0.30
Corbicula fluminea (Mollusca)	Purchased commercial culture	A: 2.47 ± 0.1 B: 4.81 ± 0.34	Controls(n = 5) Treatments(n = 5)	10 (8%)	A:0-2.89-5.41-10.42-20.45-40.45 B:0-26.62-49.59-95.44-193.51-383.1	DO = 8.53 ± 0.04 PH = 7.5 ± 0.04 T = 19.84 ± 0.22
Physa acuta (Mollusca)	Outdoor ditches in Machege	A: embryo B:	Controls(n = 5) Treatments(n = 5)	20 (13%)	A:0-0.38-0.75-1.47-2.98-5.74 B:0-3.41-6.84-13.72-27.45-52.6	DO = 8.54 ± 0.03 PH = 7.46 ± 0.01 T = 19.82 ± 0.23
Dicrotendipes pelochloris (Insecta)	Outdoor ditches in Machege	A: first instar larvae B:	Controls(n = 5) Treatments(n = 5)	20 (18%)	A:0-2.62-5.4-10.42-20.33-40.4 B:0-23.46-45.58-92.44-173.05-358.53	DO = 8.55 ± 0.05 PH = 7.44 ± 0.04 T = 20.1 ± 0.31
Neocaridina denticulata (Crustacea)	Purchased commercial culture	A:6.77 ± 0.11 B: 2.37 ± 0.04	Controls(n = 5) Treatments(n = 5)	10 (2%)	A:0-2.68-5.15-10.36-20.32-40.63 B:0-27.52-51.27-98.69-193.49-383.13	DO = 8.54 ± 0.06 PH = 7.4 ± 0.03 T = 19.86 ± 0.27
Dugesia japonica (Turbellaria)	Purchased commercial culture	A: 11.5 ± 0.3 B: not measured	Controls(n = 5) Treatments(n = 5)	10 (0%)	A:0-1.08-2.24-4.49-9.82-18.66 B:0-10.58-21.38-42.57-91.79-175.95	DO = 8.58 ± 0.07 PH = 7.42 ± 0.06 T = 20.12 ± 0.12
Hirudo nipponia (Hirudinea)	Purchased commercial culture	A: not measured B: 271.71 ± 10.27	Controls(n = 5) Treatments(n = 5)	10 (0%)	A:0-1.09-2.33-4.94-9.26-19.42 B:0-10.59-21.88-47.57-88.79-189.25	DO = 8.56 ± 0.06 PH = 7.45 ± 0.06 T = 19.76 ± 0.21

2.2 Sediment preparation and spiking

2.2.1Single species toxicity tests

All 28-d single species toxicity tests were performed in artificial sediment according to OECD guidelines (OECD 2004a, 2004b, 2007, 2010). The artificial sediment was composed of 5% finely ground sphagnum peat, 20% kaolin clay and 75% industrial sand. The wet artificial sediment moisture content was maintained at about 65.4%. These sediments contained, on average an organic carbon (OC) content of 2.43% w/w in dry sediment.

The spiking treatments included a solvent control (sediment spiked with 0.5 ml acetone/kg dry sediment), and five treatment-levels of fludioxonil. For each treatment (including controls) 5 test systems were used. The spiking was conducted from low to high concentrations (for measured geometric mean sediment exposure concentrations of each treatment in each laboratory toxicity test see Table 1) and per treatment level the dosing solution was gently mixed with a hand-held electric mixer in wet artificial sediment for 5 minutes.

2.2.2Indoor microcosm test

Approximately 4 kg wet artificial sediment was used in each microcosm as sediment compartment, and 18 microcosms were constructed for each treatment level. The microcosms experiment set up included a blank control (F1a; no acetone added), a solvent control (F1b; sediment spiked with 0.5 ml acetone/kg wet sediment) and 4 nominal treatment concentrations(respectively 2 mg fludioxonil/kg dry weight (DW) sediment (F2), 6 mg fludioxonil/kg DW sediment (F3), 18 mg fludioxonil/kg DW sediment (F4) and 54 mg fludioxonil/kg DW sediment (F5). For the measured concentrations of fludioxonil in the sediment compartment at day 0, see SI Appendix B Table 2. Per treatment level and per test system the dosing solution was gently mixed with a hand-held electric mixer in two portions of 2 kg wet sediment for 15 minutes. Then 4 kg of spiked wet sediment of a specific treatment was placed in each microcosm.

Table 2

28d-L(E)Cx values (and 95% confidence bands) for eight benthic invertebrates in laboratory toxicity tests using artificial sediment spiked with the fungicide fludioxonil. The 28d-L(E)Cx values are expressed in terms of geometric mean fludioxonil concentration during the test in the total dry sediment (mg a.s./kg DW) and in sediment pore water (µg a.s./L). The toxicity values were calculated with the MOSAIC computer program.

Taxon Endpoint	Toxicity value	Total sediment (mg a.s./kg DW)	Pore water (µg a.s./L)	Organic carbon (OC) fraction in dry sediment (mg/g OC)
Branchiura sowerbyi (Oligochaeta)				
- Reproduction (number of cocoons)	28d-EC_{10}	2.56 (1.47–4.14)	25.9 (15.3–40.8)	0.11 (0.06–0.17)
	28d-EC_{50}	7.01 (5.46–8.96)	68.4 (54.0-86.4)	0.29 (0.23–0.37)
- Relative wet weight decrease	28d-EC_{10}	5.78 (5.02–6.59)	56.4 (49.5–64.0)	0.24 (0.21–0.27)
	28d-EC_{50}	22.8 (21.2–24.6)	217 (202–233)	0.94 (0.87–1.01)
- Survival	28d-LC_{10}	19.0 (12.7–26.8)	182 (122–253)	0.78 (0.52–1.10)
	28d-LC_{50}	90.1 (59.0-166)	839 (550–1520)	3.71 (2.43–6.83)
Limnodrilus hoffmeisteri (Oligochaeta)				
- Reproduction (number of cocoons)	28d-LC_{10}	11.9 (1.19–36.4)	98.8 (9.86–321)	0.49 (0.05–1.50)
	28d-LC_{50}	35.9 (24.3–50.2)	313 (209–439)	1.48 (1.0-2.07)
- Relative rate in daily wet weight increase	28d-EC_{10}	3.64 (2.39–5.22)	31.2 (20.6–44.8)	0.15 (0.10–0.22)
	28d-EC_{50}	25.3 (22.3–29.1)	221 (194–253)	1.04 (0.92–1.20)
- Survival	28d-LC_{10}	18.3 (13.1–23.7)	158 (112–206)	0.75 (0.54–0.98)
	28d-LC_{50}	56.6 (43.1–87.3)	506 (384–786)	2.33 (1.77–3.59)
Corbicula fluminea (Mollusca)				
- Relative rate in daily length increase	28d-EC_{10}	0.402 (0.296–0.534)	3.75 (2.75–4.97)	0.017 (0.012–0.022)
	28d-EC_{50}	1.58 (1.37–1.82)	14.7 (12.7–16.9)	0.065 (0.056–0.075)
- Relative rate in daily wet weight increase	28d-EC_{10}	0.54 (0.42–0.68)	4.93 (3.86–6.19)	0.022 (0.017–0.028)
	28d-EC_{50}	1.28 (1.15–1.42)	11.83 (10.63–13.12)	0.053 (0.047–0.058)
- Survival	28d-LC_{10}	0.791 (0.390–1.42)	7.41 (3.54–13.3)	0.033 (0.016–0.058)
	28d-LC_{50}	4.44 (3.30–6.49)	40.8 (30.5–60.4)	0.18 (0.14–0.27)
Physa acuta (Mollusca)				
- Relative rate in daily length increase	28d-EC_{10}	11.6 (6.7–19.1)	109 (61.8–180)	0.48 (0.28–0.79)
	28d-EC_{50}	143 (101–219)	1390 (972–2120)	5.89 (4.16–9.01)
- Relative rate in daily wet weight increase	28d-EC_{10}	12.57 (7.46–19.11)	117.85 (69.06-181.56)	0.52 (0.31–0.79)
	28d-EC_{50}	56.23 (46.50-73.15)	534.57 (441.80-708.91)	2.31 (1.91–3.01)
- Survival	28d-LC_{10}	35.9 (24.8–40.2)	341 (235–400)	1.48 (1.02–1.65)
	28d-LC_{50}	49.4 (41.3–77.6)	465 (391–744)	2.03 (1.7-3.193)
Dicrotendipes pelochloris (Insecta)				
- Emergence	28d-EC_{10}	67.2 (33.3–80.3)	617 (308–733)	2.77 (1.37–3.31)
	28d-EC_{50}	100 (82.2–177)	912 (752–1620)	4.12 (3.83–7.28)
Neocaridina denticulata (Crustacea)				
- Relative rate in daily length increase	28d-EC_{10}	17.5 (15.5–18.6)	166 (147–176)	0.72 (0.64–0.77)

Taxon Endpoint	Toxicity value	Total sediment (mg a.s./kg DW)	Pore water (µg a.s./L)	Organic carbon (OC) fraction in dry sediment (mg/g OC)
	28d-EC_{50}	19.6 (18.9–21.5)	186 (179–204)	0.81 (0.78–0.89)
- Relative rate in daily wet weight increase	28d-EC_{10}	16.3 (12.0-18.4)	154 (113–174)	0.67 (0.49–0.76)
	28d-EC_{50}	20.4 (18.9–22.9)	193 (179–216)	0.84 (0.78–0.94)
- Survival	28d-LC_{10}	4.14 (3.11–5.31)	39.5 (29.3–50.7)	0.17 (0.13–0.22)
	28d-LC_{50}	9.24 (7.95–10.8)	87.5 (75.2–102)	0.38 (0.33–0.44)
Dugesia japonica (Turbellaria)				
- Asexual reproduction	28d-EC_{10}	9.18 (7.02–13.6)	85.9 (65.3–129)	0.38 (0.29–0.56)
	28d-EC_{50}	10.4 (9.71–14.4)	97.1 (90.9–136)	0.43 (0.40–0.59)
- Survival	28d-LC_{10}	17.2 (13.9–18.5)	163 (132–174)	0.71 (0.57–0.76)
	28d-LC_{50}	20.9 (19.0-28.5)	197 (179–267)	0.86 (0.78–1.17)
Hirudo nipponia (Hirudinea)				
- Relative wet weight decrease	28d-EC_{10}	3.24 (2.97–3.52)	31.0 (28.3–33.7)	0.13 (0.12–0.15)
	28d-EC_{50}	7.17 (6.84–7.52)	68.8 (65.6–72.2)	0.30 (0.28–0.31)
- Survival	28d-LC_{10}	11.8 (9.67–16.6)	114 (92.8–162)	0.49 (0.40–0.68)
	28d-LC_{50}	12.6 (10.0-17.2)	122 (96.2–168)	0.52 (0.41–0.71)

2.3 Design of sediment-spiked laboratory toxicity tests and microcosm test system

2.3.1 Design of sediment-spiked laboratory single species-toxicity tests

The 28-d sediment-spiked toxicity tests were all conducted in the same type of test system in the laboratories of Zhejiang University Ecological and Environmental Toxicology Research Center (see Fig. 1). The test systems consisted of 500mL glass beakers, containing 290 g wet artificial (representing approximately 100 g dry artificial sediment) and about 450 mL of aerated tap water (before introduction aerated for 72 h). Before adding overlying water the sediment was covered with a plastic sheet. The plastic sheet avoided that the sediment became suspended in the overlying water. The plastic sheet was gently removed from each test system after 10 minutes. The test systems were placed in a climate room of around 20 °C. Aeration of the water column was provided through a rubber tube and a bubble stone. pH, dissolved oxygen (DO) and temperature were measured weekly in the overlying water of each test system. The test animals were introduced in the test systems 48 h after spiking the sediment with fludioxonil.

The test performed with *D. pelochloris*, *C. fluminea*, *N. denticulata*, *P. acuta* and *D. japonicus* followed OECD test guideline 218 (OECD 2004a) as much as possible. At the start of the experiments with *D. pelochloris, C. fluminea*, *N. denticulata* and *P. acuta*, 0.5 g spinach powder was blended in the artificial sediment of each test system as initial food source. Feed was applied topically in the form of a ground paste of a 7.2 g/L algal-water mixture. Initially, 0.5 mL of this paste was supplied every three days to each test system but with the growth of the organisms the volume of the feed was appropriately increased to 1 ml on day 7 onwards. *D. japonicus* was fed with 10 living 3rd instar *D. pelochloris* larvae every three days.

At the beginning of the *H. nipponia* test, tofu of the blood of pig was fed once, and the wet weight of the *H. nipponia* after eating was recorded. No feeding occurred during the 28-day toxicity test.

The test design for the two aquatic oligochaetes was based on OECD test guideline 225 (OECD 2007). At the start of the experiment (before spiking) 0.5 g of spinach powder was mixed in the artificial sediment of each test system as a potential food source.

The endpoints measured for *C. fluminea*, *N. denticulata* and *P. acuta* were number of surviving individuals and relative rate in daily length increase. Measured endpoints for *H. nipponia* were number of surviving individuals and relative wet weight biomass decrease. The endpoints measured for *D. japonicus* were the number of surviving individuals on day 28 as well as increase in numbers due to asexual reproduction. The endpoint measured for the *D. pelochloris* was emergence of adults on day 28. In the tests with the Oligochaete worms *B. sowerbyi* and *L. hoffmeisteri* the measured endpoints were surviving individuals and the production of cocoons. In addition, for *B. sowebyi*, which in most test systems decreased in biomass during the 28d test period, relative wet weight biomass decrease was selected as measurement endpoint. For *L. hoffmeisteri*, which increased in biomass in all test systems during the 28d toxicity test, the measurement endpoint relative rate in daily biomass increase was selected. For the test to be considered valid, the mortality in control test systems should not be higher than 20% at the end of the test (USEPA 2000).

2.3.2 Indoor microcosm construction

The microcosm test system (see SI Appendix B Figs. 1 and 2) consisted of a glass fish tank with a volume of 20 L (diameter = 30 cm, height = 30 cm) containing 4 kg of (control or spiked) wet sediment and 16 L of overlying water (aerated tap water). After the introduction of the 4 kg (control or spiked) wet sediment in each microcosm test system and before introducing overlying water, the sediment was covered with a plastic sheet. The plastic sheet avoided that large amounts of sediment became suspended in the overlying water. The plastic sheet was gently removed out of each microcosm after 20 minutes and the suspended sediment was allowed to subside for 1 day. Then five 5 shoots (length of 15 cm) of the macrophyte *Myriophyllum aquaticum* were planted in each microcosm. The process of photosynthesis by the submersed parts of this macrophyte provided oxygen to the microcosm test system and habitat to some animals. Then, 4 species of freshwater algae (purchased from the Institute of Hydrobiology, Chinese Academy of Sciences), 8 species of zooplankton (provided by the Institute of Pesticides and Ecotoxicology, ZheJiang University), and 7 species of benthic animals also used in the single species toxicity tests (except *H. nipponia*) were added to each microcosm. See SI Appendix B Table 1 for the identity, properties and quantities of organisms introduced in the microcosms. The reason not to include *H. nipponia* is to avoid a too high predation pressure on the other benthic invertebrates. Furthermore note that adults of *N. denticulata* with eggs were introduced in the microcosms to facilitate potential population growth.

Throughout the test period, the microcosm systems were installed in a large water bath (shown in SI Appendix B Fig. 2a). From 0 d to 40 d, the temperature was kept at 24–26 ℃, light intensity at 3 000 lux. The daily light-dark (L - D) regime was 14 – 10 h. From day 0 onwards, 10 g of algae powder and 10 g of spinach leaf powder were added to each test system every 10 days. In addition, every 10 days, 1 mL of rotifer stock solution (20 individual /mL) and 5 adult of *Moina macrocopa* were introduced as food source.

Dissolved oxygen (DO), pH, electrical conductivity (EC) and dissolved organic carbon (DOC) were measured between 8:00 and 10:00 am at days 0, 10, 20, 30 and 40 (result see SI Appendix B Fig. 3).

2.4 Sampling of macroinvertebrates and zooplankton in microcosms

At, respectively 10d, 20d, 30d, and 40d after the start of the microcosm experiment (day 0 is the moment of seeding the microcosms with organisms) treatment-related effects on macroinvertebrates and zooplankton were studied in the test systems that were destructively sampled (three test system per treatment, including controls).

Before sampling of benthic invertebrates, a 5-L depth-integrated water sample was collected from each microcosm (see SI Appendix B Fig. 2c). The 5-L water sample was filtrated through a 25-µm mesh net and the sample concentrated was preserved in 250 µL of acetate buffered Lugol solution, the weight of the concentrate determined and stored at 4°C in the dark. All Cladocera, Copepoda (except *nauplii*) and Ostracoda individuals in the sample were counted with a Leica stereomicroscope (DM S8AP0) at a magnification of 40×. Rotifers in a known volume of the concentrated sample were identified and counted with an inverted microscope at 100× magnification. Zooplankton was identified to the lowest practical taxonomic level achievable by visual inspection of preserved animals under the microscope. Numbers of counted zooplankters in the (sub) samples were recalculated to their numbers per litre in overlying water of the microcosms.

To assess the abundance of benthic macroinvertebrates in each microcosm, the sediment was gently removed by means of stainless-steel spoon and washed over a 0.15 mm mesh sieve. Then the endobenthic macroinvertebrates were collected and identified by a dissecting microscope and counted. In addition, also the benthic macroinvertebrates present on the glass wall and macrophytes of each microcosm were sampled, counted and identified.

2.5 Fludioxonil analysis

2.5.1 Analysis of fludioxonil in single species test

The concentrations of fludioxonil in the total sediment, sediment pore water and overlying water were measured at the start (day 0) and the end (day 28) of the laboratory toxicity tests. For this, six extra test systems for each treatment (including controls) were prepared for each test species. On each sampling date (days 0 and 28) approximately 200 mL of overlying water and 100 g well-mixed wet sediment were sampled from each test system (without organism). The samples were collected in 250 mL high density polyethylene (HDPE) bottles and stored at − 20°C until qualitative analysis. These sediment and pore water samples were collected and extracted by centrifuging as described in Brock et al. (2020).

Prior to fludioxonil analysis in overlying water, the water sample was directly filtered over a 0.22 µm membrane and transferred in CG vials. All pore water samples (0.5 mL) were promptly diluted 1:1 with acetonitrile for analysis using an Agilent 1200 Series RRLC/uHPLC System (Agilent Technologies, Germany). When the sample concentration was above the highest standard of the calibration curve, the samples were further diluted with acetonitrile (50:50 v/v). All the dilutions were done in 20 mL centrifuge tubes. Then the samples were filtered over a 0.22 µm organic membrane and transferred to CG vials and analyzed. All sediment extracts were also analyzed in the same way. For a description and analysis of quality control see (SI Appendix C Tables 1–4 and SI Appendix C Figs. 1 and 2). The toxicity values of the fungicide were described in terms of (i) mg a.s./kg dry sediment, (ii) mg a.s./g OC in dry sediment and (iii) µg a. s./L sediment pore water. For this geometric mean concentrations of fludioxonil in each laboratory toxicity test were used as calculated from measurements on days 0 and 28 (see Table 2).

2.5.2 Concentration analysis of fludioxonil in microcosm experiment

Fludioxonil concentrations in sediment and pore-water were analyzed as described above in Sect. 2.5.1. Prior to fludioxonil analysis in overlying water, the water sample was directly filtered over a 0.22 µm membrane and transferred in CG vials.

To facilitate comparison of sediment exposure concentrations with experiments using other types of sediment, the concentrations of the fungicide in the spiked sediment were described in terms of (i) mg a.s./kg dry sediment, (ii) mg a.s./g OC in dry sediment and (iii) µg a.s./L sediment pore water and overlying water (see Table 3).

28-day LC_x and EC_x values (and 95% confidence bands) for the benthic test species were calculated by the MOSAIC web-interface for statistical analysis in ecotoxicology. Hazardous concentrations to 5% (HC_5) and 50% (HC_{50}) of the species tested were calculated from species sensitivity distribution (SSD) curves. These HC_p values, and their 95% confidence bands, also were estimated using the MOSAIC tool, using the median $L(E)C_{10}$ values as input. MOSAIC is available at http://pbil.univ-lyon1.fr/software/mosaic/ .

The macroinvertebrates and zooplankton abundances were ln (αx + 1) transformed before statistical analysis of microcosm data. This x stands for the abundance value, and for macroinvertebrates α = 2 and for zooplankton α = 6. This was done to down-weigh high abundance values and to approximate a normal distribution of the data (Van den Brink et al., 2000). For macroinvertebrate and zooplankton populations in the microcosm experiment NOECs ($p < 0.05$) were calculated using the LSD test by SPSS 22 (Xiao 2017). NOECs for water quality endpoints were also calculated using the LSD test, but the data was not ln-transformed. In addition, treatment-related effects on macroinvertebrate and zooplankton communities were analyzed by the Principle Response Curves (PRC) method (Van den Brink and Ter Braak 2010). We made a comparison between each treatment and the solvent control to assess the NOEC at the community level and followed by Monte Carlo permutation using the CANOCO software package (Version 5)(Lai 2013).

The population densities between blank controls and solvent-control test systems (one way ANOVA test) were compared using the SPSS 22 computer program in order to evaluate any impact of the acetone in the sediment-spiked microcosm experiment.

The Top-rice software package (NY/T 2882.2—2016) was used to predict the fludioxonil concentration in the overlying water column of South Chinese water bodies for the scenario off-crop edge-of-field ponds (Nanchang exposure scenario) after agricultural use of the fungicide in rice. For a description of the Top-rice exposure scenario and modelling procedure see SI Appendix D. To predict the fludioxonil concentration in the overlying water column of off-crop edge-of-field ponds after agricultural use of the fungicide in the crop grapes, wheat, maize and tomato the China Pesticide Surface water Exposure Model (China PSEM) procedure of the China Pesticide Information Network (ICAMA) was used. For a description of the China PSEM exposure scenario and modelling procedure see SI Appendix D.

For the rice crop, based on the Top-rice exposure scenario, the predicted environmental concentration (PEC) of fludioxonil in sediment was estimated using the following formula: $PEC_{sed} = (PEC_{sw;ch} * K_{OC})/10 * f_{OC}$. In this formula, PEC_{sed} is the PEC in sediment, $PEC_{sw;ch}$ is the long-term predicted environmental concentration in overlying water, f_{OC} is the fraction of organic carbon and K_{OC} is the partitioning coefficient between organic carbon and water of fludioxonil. This formula was proposed by (Hansen et al. 1991) and optimized by (Diepens et al. 2017; EFSA 2015). For estimating PEC_{sed} values we selected the highest 7-d and 28-d time-weighted average (TWA) concentrations as $PEC_{sw;ch}$.

Results

3.1 28-Day $L(E)C_{10}$ and $L(E)C_{50}$ data for the benthic species tested

All 28d-$L(E)C_{10}$ and 28d-$L(E)C_{50}$ values (and 95% confidence intervals) of the 8 benthic macroinvertebrates and the measured endpoints are shown in Table 2. The basic data underlying these toxicity estimates are presented in SI Appendix A Tables 1–8.

3.2 Species sensitivity distributions (SSDs)

SSDs were constructed with 28-d EC_{10} and 28-d LC_{10} values as input. For each species and the different sublethal endpoints measured we selected the lowest 28-d EC_{10} value. The endpoints that resulted in the lowest EC_{10} were reproduction for *B. sowerbyi* and *D. japonica*, relative rate in daily biomass increase for *L. hoffmeisteri*, relative rate in daily length increase for *C. fluminea*, *P. acuta* and *N. denticulata*, emergence for *D. pelochloris* and relative biomass decrease for *H. nipponia*. The 28-d EC_{10} for the endpoint emergence of *D. pelochloris* was also considered to be the 28-d LC_{10} value for this species, since after 28 days no surviving larvae could be found in the sediment anymore. The 28-d LC_{10} values for all species were highly comparable, since they all refer to the survival endpoint. The position of the different benthic species within the SSD curves is presented in Fig. 2.

The available 28d-EC_{10} estimates expressed in terms of fludioxonil concentration in total dry sediment and in pore water showed the following order: *C. fluminalis* < *B. sowerbyi* < *H. nipponia* < *L. hoffmeisteri* < *D. japonicus* < *P. acuta* < *N. denticulata* < *D. pelochloris* (Fig. 2A&C).

The available 28d-LC_{10} estimates expressed in terms of mg a.s./kg dry sediment, showed the following order: *C. fluminalis* < *N. denticulata* < *D. japonicus* < *H. nipponia* < *L. hoffmeisteri* < *B. sowerbyi* < *P. acuta* < *D. pelochloris*(Fig. 2B). Expressing the 28d-LC_{10}'s in terms of µg/L pore water, the sensitivity order of species slightly shifted in that *H. nipponia* and *L. hoffmeisteri* were more sensitive than *D. japonicus* (Fig. 2D).

3.3 Hazardous concentrations

The median HC_5 (and its 95% confidence band) for the benthic invertebrates tested on basis of total sediment concentration was 0.57 (0.15–3.1) mg a.s./kg for sublethal endpoints (Fig. 2A) while that was 1.5 (0.48–6.7) mg a.s./kg for the lethal endpoint (Fig. 2B). In term of pore water concentration (Fig. 2C, 2D), the median HC_5 (and 95% confidence band) was 5.4 (1.5–28) µg a.s./L for sublethal endpoints and 14 (4.2–64) µg a.s./L for mortality. HC_{50} values were a factor of 8–10 higher than corresponding HC_5's.

3.4 Microcosm exposure concentrations

Concentrations in the sediment compartment as mg a.s./kg dry sediment are given in Fig. 3A. As expected, higher concentrations were measured with increasing treatment-level. A slow decline in fludioxonil concentration in the total sediment was observed between day 0 and day 40 but in systems that received the highest treatments the decline was somewhat faster. Geometric mean concentrations in mg a.s./kg dry weight for different time periods are

reported in Table 3. Initially (period 0–10 d) geometric mean concentrations were higher at all treatment-levels but differences between different time-periods (0–10 d; 0–20 d; 0–30 d and 0–40 d) were small for all treatments, indicating that sediment-dwelling (endobenthic) invertebrate populations experienced chronic exposure to fludioxonil. The fludioxonil exposure patterns expressed in terms of µg a.s./g OC in dry sediment are proportional to those for total sediment concentration expressed in terms of mg a.s./kg dry sediment (see Fig. 3A and D; Table 3).

Table 3:Geometric mean concentrations of fludioxonil in total sediment, pore water, organ carbon fraction in sediment and overlying water (water column) per treatment-level for different time periods in the indoor sediment-spiked microcosm experiment.

Treatment level (spiked sediment)	Total concentration in mg/kg dry sediment				Total concentration in mg/g organic carbon (OC)				Pore water concentration in µg/L				Water column concentration (µg/L)			
	0d-10d	0d-20d	0d-30d	0d-40d	0d-10d	0d-20d	0d-30d	0d-40d	0d-10d	0d-20d	0d-30d	0d-40d	0d-10d	0d-20d	0d-30d	0d-40d
F1a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F1b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F2	2	2	1.97	1.95	0.08	0.08	0.08	0.08	10.94	10.97	10.78	10.69	0.57	0.59	0.6	0.62
F3	5.99	5.89	5.82	5.77	0.25	0.24	0.24	0.24	33.44	32.88	32.46	32.18	1.85	1.93	2.02	2.17
F4	17.45	17.23	17.06	16.94	0.72	0.71	0.7	0.7	109.12	107.72	106.66	105.96	5.69	5.89	6.11	6.29
F5	49.25	47.83	46.45	45.65	2.03	1.97	1.91	1.88	435.1	422.52	410.35	403.31	22.82	23.2	23.68	23.88

The sediment pore water concentrations of fludioxonil are reported in Fig. 3B and Table 3. During days 0–40 a slow decline in fludioxonil pore water concentration was observed in the microcosms. The geometric mean of pore water concentrations for different time periods (0–10 d; 0–20 d; 0–30 d and 0–40 d) illustrate a long-term chronic exposure regime for typical sediment-dwelling organisms (Table 3). Fludioxonil in pore water contributed 0.55%-0.88% of the fludioxonil in dry sediment.

Concentrations of fludioxonil in the overlying water of the microcosm test system are presented in Fig. 3C. Concentrations in the overlying water slightly increased in time at all treatment levels. Differences in geometric mean concentrations between different time-periods were relatively small within treatments, indicating that freshwater zooplankton populations experienced chronic exposure to fludioxonil (Table 3).

3.5 Responses of benthic macroinvertebrate populations to fludioxonil exposure

Principle Reponses Curves (PRC), a multivariate analysis technique, and Monte Carlo permutation of the benthic macroinvertebrates sampled in the microcosms demonstrated clear community-level effects (Table 4, Fig. 3). The treatment-related response of the benthic macroinvertebrate community in the total test system is significant ($p = 0.002$). Statistically significant differences in community structure between solvent controls and treatments for the total system were observed for the F3 (sampling days 10 and 20), F4 (day 40) and F5 (day 30) treatments (Table 4).

Table 4: NOECs (LSD test, $p \leq 0.05$; expressed in terms of treatment-level) for the community and populations of macroinvertebrate taxa in the total microcosm test system. Exposure concentrations in sediment and water for the different treatments are reported in Table 3.

Scientific name	Sampling day after start experiment				Note
	10d	20d	30d	40d	
Community (PRC)	F2	F2	F4	F3	
Neocaridina denticulata	F2↓	F2↓	F2↓	F3↓	SI B Fig 4
Dicrotendipes pelochloris	F4↓	F4↓	-	-	SI B Fig 4
Physa acuta	F4↓	F4↓	F4↓	F4↓	SI B Fig 4
Corbicula fluminea	F4↓	F3↓	F2↓	F3↓	SI B Fig 4
Limnodrilus hoffmeisteri	F3↓	F4↓	F4↓	F3↓	SI B Fig 4
Branchiura sowerbyi	F3↓	-	-	F3↓	SI B Fig 4
Dugesia japonica	F3↓	F3↓	F4↓	F3↓	SI B Fig 4

– = NOEC highest treatment level,↓=decrease in abundance.

The high positive species score ($bk > 1.5$) for *N. denticulata* in the PRC diagram (Fig. 4) indicates that the decline in abundance of this crustacean correlated best with the community response. The lowest species score (*bk* of approximately 0) was observed for the chironomid *D. pelochloris* (Fig. 4). 63.57% of the total variance of the benthic macroinvertebrate dataset is explained by treatment and 28.97% by the factor time. The response in the PRC of the benthic

macroinvertebrate community in the total test system is significant ($p = 0.002$) and of the overall variance 92.9% is presented on the first canonical axis ($p = 0.002$).

In Table 4, taxa of benthic macroinvertebrates are presented for which reliable population-level NOECs could be calculated (in total 7 taxa). In our microcosm experiment, the crustacean *N denticulata* showed the most sensitive treatment-related response with a NOEC at treatment-level F2 on sampling days 10, 20 and 30 (Table 4, SI Appendix B Fig. 4). Also the bivalve *C. fluminea* showed a NOEC as low as treatment-level F2 on sampling day 30 (Table 4, SI Appendix B Fig. 4). Taxa with a NOEC observed at treatment-level F3 were the oligochaetes *L. hoffmeisteri* and *B. sowerbyi* and the triclad *D. japonica* (Table 4, SI Appendix B Fig. 4). Long-term treatment-related declines were observed for *P. acuta* at the highest treatment level (F5) (Table 4, SI Appendix B Fig. 4). Also *D. pelochloris* showed a treatment-related decline at treatment-level (F5) but only initially (Table 4, SI Appendix B Fig. 4).

3.6. Responses of zooplankton to fludioxonil exposure

The PRC for the zooplankton community is presented in Fig. 5. Monte Carlo permutation of the zooplankton sampled in the microcosms demonstrated clear community-level effects for the F4 and F5 treatments on all sampling days (Table 5). In the PRC 52.67% of the total variance of the zooplankton dataset is explained by treatment and 28.3% by the factor time. The PRC is significant ($p = 0.002$) and of the overall variance 86.2% is presented on the first canonical axis ($p = 0.002$).

Table 5
NOECs (LSD test, $p \leq 0.05$; expressed in terms of treatment level) for the community and populations of zooplankton taxa in the overlying water of the microcosms. Exposure concentrations in the water column for the different treatments are reported in Table 3.

Scientific name	Sampling day after start experiment				Note
	10d	20d	30d	40d	
Community (PRC)	F3	F3	F3	F3	
Daphnia carinata	F3↓	F4↓	F3↓	F3↓	SI B Fig. 5
Moina macrocopa	F3↓	F3↓	F3↓	F3↓	SI B Fig. 5
Scapholeberis mucronata	F3↓	F3↓	F4↓	-	SI B Fig. 5
Eucyclops serrulatus	-	-	-	-	SI B Fig. 5
Brachionus calyciflorus	-	-	-	-	SI B Fig. 5
Polyarthra sp	-	-	-	-	SI B Fig. 5
Dolerocypris sinensis	F4↓	F3↓	F3↓	F3↓	SI B Fig. 5
Cypridopsis sp.	F4↓	F3↓	F3↓	-	SI B Fig. 5
– = NOEC highest treatment level,↓=decreaseinabundance.					

The high positive species scores ($bk \geq 1$) of the cladocerans *D. carinata*, *M. macrocopa, S. mucronate* and the ostracods *Cypridopsis* sp. and *D. sinensis* in the PRC diagram (Fig. 5) indicate that their decline in abundances correlated best with the community response. At the population-level the lowest NOEC observed for these zooplankton taxa was treatment-level F3 (Table 5; SI Appendix B Fig. 5). The copepod *E. serrulatus* and rotifers *B. calyciflorus* and *Monostyla* sp. with a *bk* score of approximately 0, did not reveal a clear treatment-related response (Table 5; SI Appendix B Fig. 5).

Discussion

4.1 Sediment-spiked laboratory single-species tests

The naturally vegetated lands in most of the coastal regions of China are experiencing an unprecedented rate of conversion to agricultural, urban, and industrial land uses (Wang et al. 2012; Zhang et al. 2010). It is reported that benthic macroinvertebrate assemblages are good indicators for assessing ecological quality in surface waters of river basins in China (Liu 2016). For this reason we selected benthic test species for our sediment-spiked toxicity tests that are more or less typical for edge-of-field surface waters in the Yangtze river-delta. In our sediment-spiked single-species toxicity tests with fludioxonil, the Asian clam *C. fluminea* appeared to be the most sensitive benthic species tested (Fig. 2). *C. fluminea* also is an economically important aquatic species in China and has been used as a test organism in many environmental studies (Chen 2013). It is a suspension feeder that filter-feeds on unicellular algae, bacteria and detrital particles.

The SSD curves presented in Fig. 2 confirm the biocidal property of the fungicide fludioxonil in that the toxicity values of different taxonomic groups result in a nice fit of the SSD curve. This observation is in line with results of a study in The Netherlands on sediment toxicity of fludioxonil to 8 other species of benthic macroinvertebrates, including two clams, two oligochaetes, two crustaceans and two insects (Brock et al. 2020). From SSD curves constructed with 28d-EC_{10} values, Brock et al. (2020) calculated HC_5 value of respectively 3.1 (1.5–7.9) mg a.s./kg dry sediment and 35 (14–110) µg a.s./L pore water. These 28d-EC_{10} based HC_5 values are approximately a factor of 6 higher than those in our study (Fig. 2), although the 95% confidence intervals of our HC_5's overlap with those of Brock et al. (2020). The fact that our data overall resulted in lower 28d-EC_{10} based HC_5 values might have several reasons. The most obvious reasons likely concern differences in species and life-stages tested, assessment endpoint selected and differences in sediment properties. The sublethal assessment endpoints selected for HC_5 calculation in the Brock et al. (2020) study were 'relative rate in daily dry weight biomass increase' for the six non-

insect taxa and 'emergence' for the two insect taxa. In our study the selected sublethal assessment endpoints for HC_5 calculation were more diverse, including reproduction, length increase, emergence and wet weight endpoints (see Table 2). Another difference is that in all our single-species tests artificial sediment was used, while the sediment-spiked toxicity tests of Brock et al. (2020) used field-collected sediment. This may have caused differences in bioavailability of fludioxonil in the sediments used and/or in environmental conditions that affect growth and sensitivity of test organisms. Studies that compared the sensitivity of the standard benthic test species *Chironomus riparius* between tests that used artificial and field-collecting sediment reported a higher sensitivity in artificial sediment spiked with pyrethroids (e.g. Fleming et al. 1998; Äkerblom et al. 2008) but a lower toxicity in artificial sediment spiked with the benzoyl insecticide lufenuron (Brock et al. 2018). Results of sediment-spiked toxicity tests with the oligochaete *Lumbriculus variegatus* and fludioxonil revealed an 28d-EC_{10} of 107 (53–225) µg/L pore water in artificial sediment (unpublished data Wageningen Environmental Research) and of 208 (123–313) µg/L pore water in field-collected sediment (Brock et al. 2020), suggesting a somewhat higher toxicity in artificial sediment although the 95% confidence intervals overlapped. Another difference between our study and that of Brock et al. (2020) is that we used fludioxonil-spiked sediment characterized by an ageing period of 2 days before introducing test organisms, while the sediment-spiked toxicity tests of Brock et al. (2020) adopted an ageing period of 7–8 days when the sediment was not in the freezer. Brock et al (2020) stored the fludioxonil-spiked sediment in a freezer for approximately a year before use in toxicity tests and it was assumed that ageing processes were stopped in the freezer at temperatures of − 20°C. The relatively short ageing period in our tests may have caused an initial higher bioavailability of fludioxonil in the total sediment and sediment pore water, compared to that of the sediment used by Brock et al. (2020). A decline in bioavailability of persistent organic chemicals due to ageing processes is reported in the scientific literature (e.g. Landrum et al., 1992; Alexander, 2000; Choi et al., 2012; Nybom et al., 2015).

4.2 Fate of fludioxonil and treatment-related responses in laboratory microcosms

In our sediment-spiked indoor microcosm experiment, the fludioxonil concentration in the sediment compartment slightly decreased, particularly during the first 30 days, while exposure concentrations slightly increased in overlying water in that period (Fig. 3). The process of reaching equilibrium between the spiked-sediment compartment and overlying water apparently took at least 30 days. In addition, the rate of degradation/dissipation of fludioxonil was slow in the microcosms. In all treatments, the geometric mean exposure concentrations in sediment and overlying water did not deviate substantially between different exposure periods, indicating a chronic exposure regime (Table 3). Similar exposure dynamics of fludioxonil were reported for sediment-spiked outdoor microcosms in The Netherlands by Yin et al. (2018).

In our indoor sediment-spiked microcosm experiment, and based on calculated NOECs for population abundance, the sensitivity of benthic macroinvertebrate populations was more or less as follows: *N. denticulata* < *C. fluminea* < *D. japonica* < *L. hoffmeisteri* < *B, sowerbyi* < *P. acuta* < *D. pelochloris* (Table 4). This order of sensitivity is in line with the order of sensitivity observed in the sediment-spiked single species tests with these species for the endpoint 28d-LC_{10} (see Fig. 2B), except that in the microcosms *N. denticulata* was more sensitive than *C. fluminea*. Note that the sublethal endpoints measured in the single-species tests were not assessed when sampling the macroinvertebrates in the indoor microcosms. Nevertheless, the abundance data from the microcosm test apparently correlate best with mortality data from the single-species tests. The order of sensitivity of benthic macroinvertebrate populations described above is partly reflected in the b_k scores of the PRC diagram presented in Fig. 3. A possible explanation why *N. denticulata* was more sensitive than *C. fluminea* in the microcosm test system compared to the single species tests might be that the microcosm test system was seeded with *N. denticulata* individuals that carried eggs while that was not the case for the individuals selected for the single-species test.

The population-level responses of macroinvertebrates observed in our indoor sediment-spiked microcosm experiment treated with fludioxonil can be compared with treatment-related responses of benthic macroinvertebrates of the sediment-spiked outdoor microcosm experiment treated with the same fungicide (Yin et al., 2018). They observed that populations of oligochaete worms were more sensitive than clams, snails, crustaceans and insects. In the outdoor sediment-spiked microcosm experiment, NOECs of 14.2 mg a.s./kg dry sediment, 88.8 µg a.s./L pore water and 5.6 µg a.s./L overlying water (values based on 0-28d geomean concentrations) were calculated for both the most sensitive benthic macroinvertebrate (the oligochaete *Dero digitata*) and the total benthic macroinvertebrate community (Yin et al., 2018). In our indoor sediment-spiked microcosm test the most sensitive benthic macroinvertebrate was the shrimp *N. denticulata* with NOECs as low as 1.97 mg a.s./kg dry sediment, 10.97 µg a.s./L pore water and 0.6 µg a.s./L overlying water (values based on 0-30d geomean concentrations). In our microcosm study, the PRC analysis revealed similar NOECs as observed for *N. denticulata* (Table 4). The lower NOEC values for benthic macroinvertebrates observed in our experiment might be explained by the fact that we used artificial sediment and focused on short-term to intermediate-term responses (observation period 10–40 days after construction microcosm experiment) and that Yin et al. (2018) used field-collected sediment and focused on intermediate-term to long-term responses (observation period 28–84 days after construction microcosm experiment). It seems reasonable to assume that the bioavailability of fludioxonil in the sediment compartment decreased after a longer ageing period (also see Sect. 4.1).

In our microcosm experiment also the treatment-related effects of fludioxonil on zooplankton was investigated. Since zooplankters are typical pelagic organisms, their responses can best be evaluated on basis of exposure concentrations in overlying water. Both for the total zooplankton community and for populations of *D. carinata*, *M. macrocopa*, *S. mucronata*, *D. sinensis* and *Cypridopsis* sp., NOECs a low as 2.02 µg/L overlying water (value based on 0-30d geomean concentrations) could be calculated (Table 5; see also SI Appendix B Fig. 5). In the outdoor sediment-spiked microcosm experiment in The Netherlands treated with fludioxonil (Yin et al., 2018), the NOEC of the most sensitive zooplankton population (the cladoceran *Diaphanosoma brachyurum*) was 1.6 µg/L overlying water (value based on 0-28d geomean concentrations) while that was 5.6 µg/L overlying water for the total zooplankton community. It thus appears that the lowest long-term NOECs observed for zooplankton were very similar between our indoor microcosm test and that of Yin et al. (2018), at least when expressed in terms of exposure concentrations in the water column, so differences in sediment bioavailability of fludioxonil due to differences in ageing processes hardly play a role for pelagic zooplankton species. Nevertheless, also some pronounced differences in treatment-related responses to fludioxonil exposure could be observed between the two microcosm experiments. The diversity of zooplankton species in the outdoor microcosm experiment of Yin et al. (2018) was much higher while also several rotifer populations showed pronounced treatment-related declines in contrast to our study.

4.3 Potential environmental risks of fludioxonil in edge-of-field ponds in China

There are more than 100 products containing fludioxonil registered in China (http://www.chinapesticide.org.cn/). Based on the observed treatment-related effects in our sediment-spiked single species tests and the indoor microcosm experiment and PEC calculations according to the Top-rice and China PSEM modelling procedures, a provisional risk assessment of exposure to fludioxonil for benthic macroinvertebrates and pelagic zooplankton in edge-of-field ponds in China can be conducted. In Tables 6, 7 and 8 this is done for the crops rice, grapes, wheat, maize and tomato. The 0–7 day PEC_{sed} values and 0–28 PEC_{sed} values calculated for fludioxonil in the total sediment ranged between respectively 0.037–120 µg/kg dry sediment and 0.037–40 µg/kg dry sediment (Tables 6 and 7). On basis of concentrations in overlying water the 0–7 day PEC_{sw} and 0–28 day PEC_{sw} values ranged between respectively 0.084–0.35 µg/L and 0.067–0.118 µg/L (Table 8).

Table 6

The sediment compartment risk quotient (RQ) of fludioxonil application in several crops for benthic macroinvertebrates in off-crop edge-of-field ponds when using the SSD approach in the effect assessment. The PEC for the sediment compartment (in mg fludioxonil/kg dry sediment) was calculation for the edge-of-field pond scenario according to the Top-rice modelling procedure for rice and the China Pesticide Surface water Exposure Model (China PSEM) procedure of the China Pesticide Information Network (ICAMA) for other crops. The HC_5 was derived from the SSD constructed with 28d-EC_{10} values expressed in terms of mg fludioxonil/kg dry sediment (see Fig. 2A). The application of an AF of 3 followed the recommendations by EFSA (2015). RQ = PEC/HC_5*AF

Crop	Application rate per treatment(kg as/ha)	Community	Assessment factor (AF)	HC_5	0-7d geomean PEC_{sed}	RQ1	0-28d geomean PEC_{sed}	RQ2
Rice*	0.026	Benthic macroinvertebrates	3	570 µg/kg	120 µg/kg	0.63	40 µg/kg	0.21
Grapes	0.126	Benthic macroinvertebrates	3	570 µg/kg	0.198 µg/kg	0.00	0.12 µg/kg	0.00
Wheat	0.0088	Benthic macroinvertebrates	3	570 µg/kg	0.041 µg/kg	0.00	0.041 µg/kg	0.00
Maize	0.0088	Benthic macroinvertebrates	3	570 µg/kg	0.037 µg/kg	0.00	0.037 µg/kg	0.00
Tomato	0.15	Benthic macroinvertebrates	3	570 µg/kg	0.0488 µg/kg	0.00	0.0479 µg/kg	0.00

*For rice the PECs in sediment were calculated by the formula: $PEC_{sed} = (PEC_{sw;ch} * K_{OC})/10 * f_{OC}$; where $PEC_{sw;ch}$ is the long-term PEC in overlying water, PEC_{sed} is PEC in sediment, f_{OC} is the fraction of organic carbon and K_{OC} is the partitioning coefficient between organic carbon and water of fludioxonil; K_{OC} = 145,600.

Table 7

The sediment compartment risk quotient (RQ) of fludioxonil application in several crops for benthic macroinvertebrates and zooplankton in off-crop edge-of-field ponds when using the model ecosystem approach in the effect assessment. The PEC for the sediment compartment (in mg fludioxonil/kg dry sediment) was calculation for the edge-of-field pond scenario according to the Top-rice modelling procedure for rice and the China Pesticide Surface water Exposure Model (China PSEM) procedure of the China Pesticide Information Network (ICAMA) for other crops. The NOEC of the most sensitive population was derived from the indoor microcosm experiment described in this paper (see Tables 3, 4 and 5). RQ = PEC/NOEC*AF

Crop	Application rate per treatment (kg as/ha)	Community	Assessment factor (AF)	NOEC (geomean 0-30d)	0-7d geomean PEC_{sed}	RQ1	0-28d geomean PEC_{sed}	RQ2
Rice*	0.026	Benthic macroinvertebrates	2	1970 µg/kg (F2)	120 µg/kg	0.12	40 µg/kg	0.04
		Zooplankton	2	5820 µg/kg (F3)	120 µg/kg	0.04	40 µg/kg	0.014
Grapes	0.126	Benthic macroinvertebrates	2	1970 µg/kg (F2)	0.198 µg/kg	0.00	0.12 µg/kg	0.00
		Zooplankton	2	5820 µg/kg (F3)	0.198 µg/kg	0.00	0.12 µg/kg	0.00
wheat	0.0088	Benthic macroinvertebrates	2	1970 µg/kg (F2)	0.041 µg/kg	0.00	0.04 µg/kg	0.00
		Zooplankton	2	5820 µg/kg (F3)	0.041 µg/kg	0.00	0.04 µg/kg	0.00
Maize	0.0088	Benthic macroinvertebrates	2	1970 µg/kg (F2)	0.037 µg/kg	0.00	0.037 µg/kg	0.00
		Zooplankton	2	5820 µg/kg (F3)	0.037 µg/kg	0.00	0.037 µg/kg	0.00
Tomato	0.15	Benthic macroinvertebrates	2	1970 µg/kg (F2)	0.0488 µg/kg	0.00	0.0479 µg/kg	0.00
		Zooplankton	2	5820 µg/kg (F3)	0.0488 µg/kg	0.00	0.0479 µg/kg	0.00

*For rice the PECs in sediment were calculated by the formula: $PEC_{sed} = (PEC_{sw;ch} * K_{OC})/10 * f_{OC}$; where $PEC_{sw;ch}$ is the long-term PEC in overlying water, PEC_{sed} is the PEC in sediment, f_{OC} is the fraction of organic carbon and K_{OC} is the partitioning coefficient between organic carbon and water of fludioxonil; K_{OC} = 145,600.

Table 8: The overlying water compartment risk quotient (RQ) of fludioxonil application in several crops for benthic macroinvertebrates and zooplankton in off-crop edge-of-field ponds. The PEC for the overlying water compartment (in µg fludioxonil/L) was calculation for the edge-of-field pond scenario according to the Top-rice modelling procedure for rice and the China Pesticide Surface water Exposure Model (China PSEM) procedure of the China Pesticide Information Network (ICAMA) for other crops. The NOEC of the most sensitive population was derived from the indoor microcosm experiment described in this paper (see tables 3, 4 and 5). RQ = PEC/NOEC*AF

Crop and/ or situation	Application rate per treatment(kg as/ha)	Community	Assessment factor (AF)	NOEC (geomean 0-30d)	0-7d geomean PECsw	RQ1	0-28d geomean PECsw	RQ2
Rice	0.026	Benthic macroinvertebrates	2	0.6 µg/L (F2)	0.35 µg/L	1.17	0.1 µg/L	0.33
		Zooplankton	2	2.02 µg/L (F3)	0.35 µg/L	0.34	0.1 µg/L	0.09
Grapes	0.126	Benthic macroinvertebrates	2	0.6 µg/L (F2)	0.096 µg/L	0.32	0.091 µg/kg	0.3
		Zooplankton	2	2.02 µg/L (F3)	0.096µg/L	0.095	0.091 µg/L	0.09
Wheat	0.0088	Benthic macroinvertebrates	2	0.6 µg/L (F2)	0.153 µg/L	0.51	0.101 µg/L	0.34
		Zooplankton	2	2.02 µg/L (F3)	0.153 µg/L	0.15	0.101 µg/L	0.1
Maize	0.0088	Benthic macroinvertebrates	2	0.6 µg/L (F2)	0.084 µg/L	0.28	0.067 µg/L	0.22
		Zooplankton	2	2.02 µg/L (F3)	0.084 µg/L	0.08	0.067 µg/L	0.066
Tomato	0.15	Benthic macroinvertebrates	2	0.6 µg/L (F2)	0.166 µg/L	0.55	0.118 µg/L	0.39
		Zooplankton	2	2.02 µg/L (F3)	0.166 µg/L	0.16	0.118 µg/L	0.12

By applying an assessment factor (AF) of 3 to the HC_5 (following EFSA, 2015) derived from the SSD constructed with 28d-EC_{10} values of benthic macroinvertebrates, the calculated risk quotients (RQs) for sediment dwelling macroinvertebrates are highest for the rice crop, but < 1 for all crops, indicating 'acceptable' risks of sediment exposure to fludioxonil (Table 6).

By applying an AF of 2 (following EFSA, 2013) to the lowest population-level NOEC from the microcosm experiment, and that was based on total sediment concentration, the calculated risk quotients (RQs) for both benthic macroinvertebrates and pelagic zooplankton again are highest for the rice crop, but < 1 for all crops, indicating 'acceptable' risks of sediment exposure to fludioxonil (Table 7).

By applying an AF of 2 (following EFSA, 2013) to the lowest population-level NOEC from the microcosm experiment, and that was based on exposure concentrations in overlying water, the calculated risk quotient (RQ) for benthic macroinvertebrates and the rice crop only was somewhat larger than 1 if the 0-7d PEC_{sw} value was used in the quotient, suggesting that some risks on epibenthic macroinvertebrates in particular cannot be excluded (Table 8). Note, however, that these epibenthic macroinvertebrates also experienced a higher exposure concentration via the sediment compartment, while this was not the case for pelagic zooplankton that mainly experience exposure via the overlying water compartment. For all other crops and wen using the 0-7d PEC_{sw} the RQs were always < 1 for both benthic macroinvertebrates and zooplankton, indicating 'acceptable' risks of water exposure to fludioxonil. When using the 0-28d PEC_{sw} in the RQ also for the benthic macroinvertebrates and fludioxonil application in rice, the RQ was < 1, suggesting 'acceptable risks' (Table 8).

Conclusions

Our experiments allow a comparison in effect assessment of sediment-exposure to the fungicide fludioxonil for benthic macroinvertebrates between the SSD approach and the model ecosystem approach (indoor microcosm experiment). In following the procedure proposed by the European Food Safety Authority (EFSA, 2015), the regulatory acceptable concentration for benthic macroinvertebrates in the sediment compartment based on the SSD approach (HC_5/3 = 0.19 mg fludioxonil/kg dry sediment) appears to be lower than that of the model ecosystem approach (lowest population level NOEC/2 = 0.99 mg fludioxonil/kg dry sediment). This is consistent with the principle of the tiered approach that a lower tier should be more conservative than a higher tier.

The results of our experiments can be compared with similar experiments conducted with fludioxonil in The Netherlands (Yin et al., 2018; Brock et al. 2020). It appears that our sediment-spiked single species toxicity tests with benthic macroinvertebrates resulted in an approximately 6 times lower median HC_5 than that of the study in The Netherlands, most likely due to different types of sediment used (artificial versus field-collected) and different ageing periods and sediment storage adopted after fludioxonil-spiking before starting the toxicity tests, in this way affecting bioavailability of fludioxonil. Also differences in benthic species and effect endpoints selected may have contributed to this difference. Nevertheless, our study results do not allow to conclude that Chinese benthic macroinvertebrates in general are more sensitive to sediment-exposure of fludioxonil than Dutch benthic macroinvertebrates. For an appropriate comparison similar external test conditions (e.g. artificial sediment; similar ageing periods and sediment storage practices) and effect assessment endpoints need to be adopted.

We observed in the indoor microcosm experiment that fludioxonil persisted in the sediment compartment and showed a very slow dissipation, resulting in long-term exposure regimes in both the sediment and overlying water compartments. This was also observed in the outdoor microcosm experiment conducted in The Netherlands (Yin et al., 2018). Interestingly, the lowest NOEC observed for zooplankton species in our indoor microcosm test system and expressed in

terms of exposure concentration in overlying water (1.85–2.02 µg fludioonil/L) was very similar to that in the Dutch outdoor microcosm test system (1.6–2.3 µg fludioonil/L; Yin et al., 2018). As explained above, the larger difference in test results for benthic-macroinvertebrates between our study and the Dutch study in part may be explained by a higher bioavailability of fludioxonil in the sediment of our experiments (artificial sediment and shorter ageing period), while this is not an issue for pelagic organisms in the water column.

Declarations

CRedit authorship contribution statement:

J Sun investigated, analysed and draftedoriginal draft; XH Yin and J Sun designed the experiments; PF Xiao, GN Zhu and K Zhang assisted the experiments; XH Yin and Theo Brock analysed most of data; XH Yin andJ Sun wrote the manuscript; XH Yin and Theo Brock contributed substantially to writing-review and editing.

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Consent to Participate (Ethics)

Written informed consent for publication was obtained from all participants.

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Figures

Figure 1

Left: A Schematic diagram of the experimental system used. (A, rotary scavenging valve; B, overlying water level; C, upside down plastic bowl used in tests to monitor emerging insects and to limit evaporation of water; D, bubble stone; E, sediment; Arrow, direction of air flow.) Right: The experimental systems in incubator.

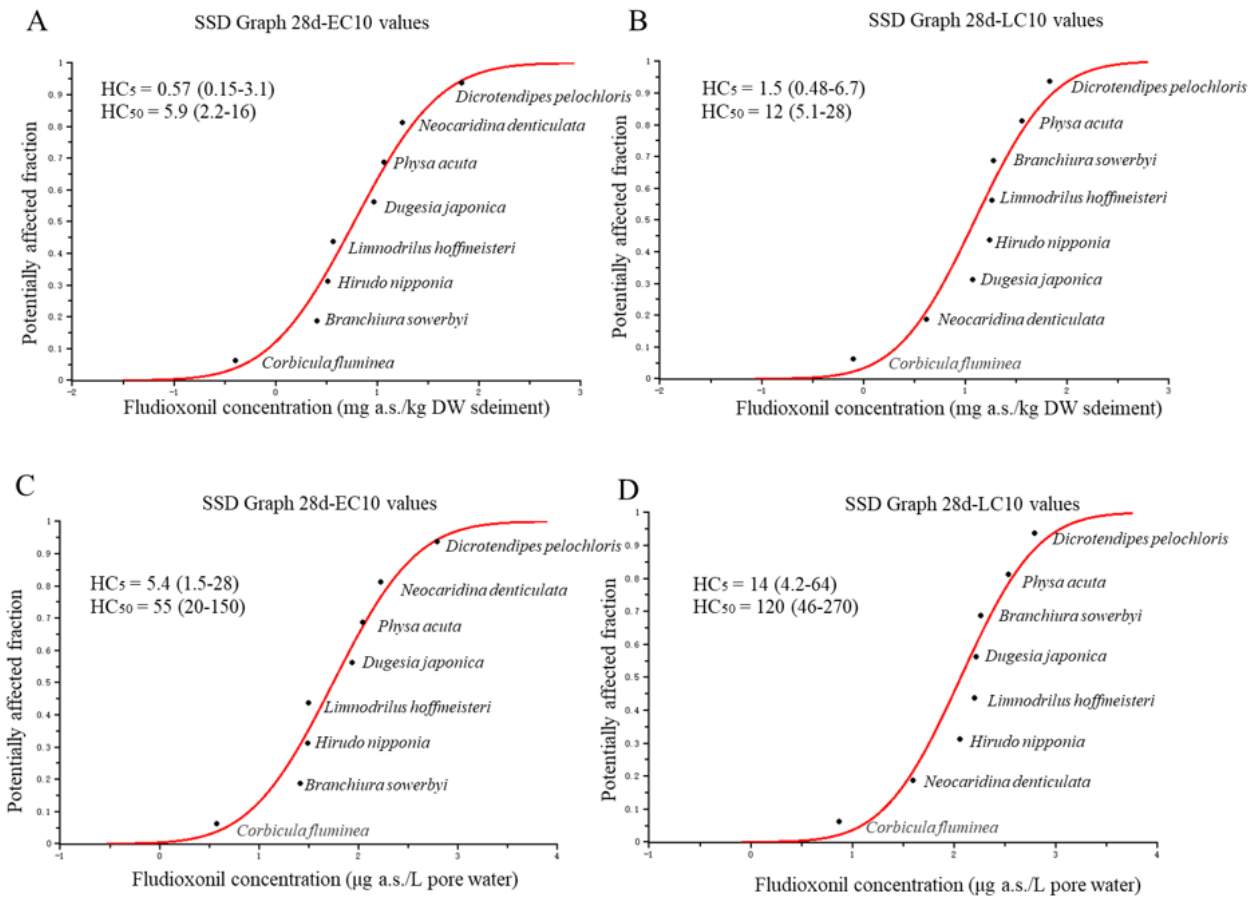

Figure 2

Species sensitivity distributions (SSDs) constructed with 28d-EC10 values (panels A, C) or 28d-LC10 values (panels B, D) for 8 different benthic invertebrates and derived from laboratory sediment toxicity tests using artificial sediment, spiked with the fungicide fludioxonil. The 28d-L(E)C10 values are expressed in terms of total sediment concentration (mg a. s./kg DW; panels A and B) and in sediment pore water concentration (µg a. s./L; panels C and D). Input data are detailed in Table 2. As 28d-EC10 input data for panels A and C, the most sensitive sublethal endpoint of 8 benthic invertebrates is selected. Hazardous concentrations to 5% (HC 5) and 50% (HC 50) of the species and their 95% confidence bands calculated with MOSAIC are presented in each panel.

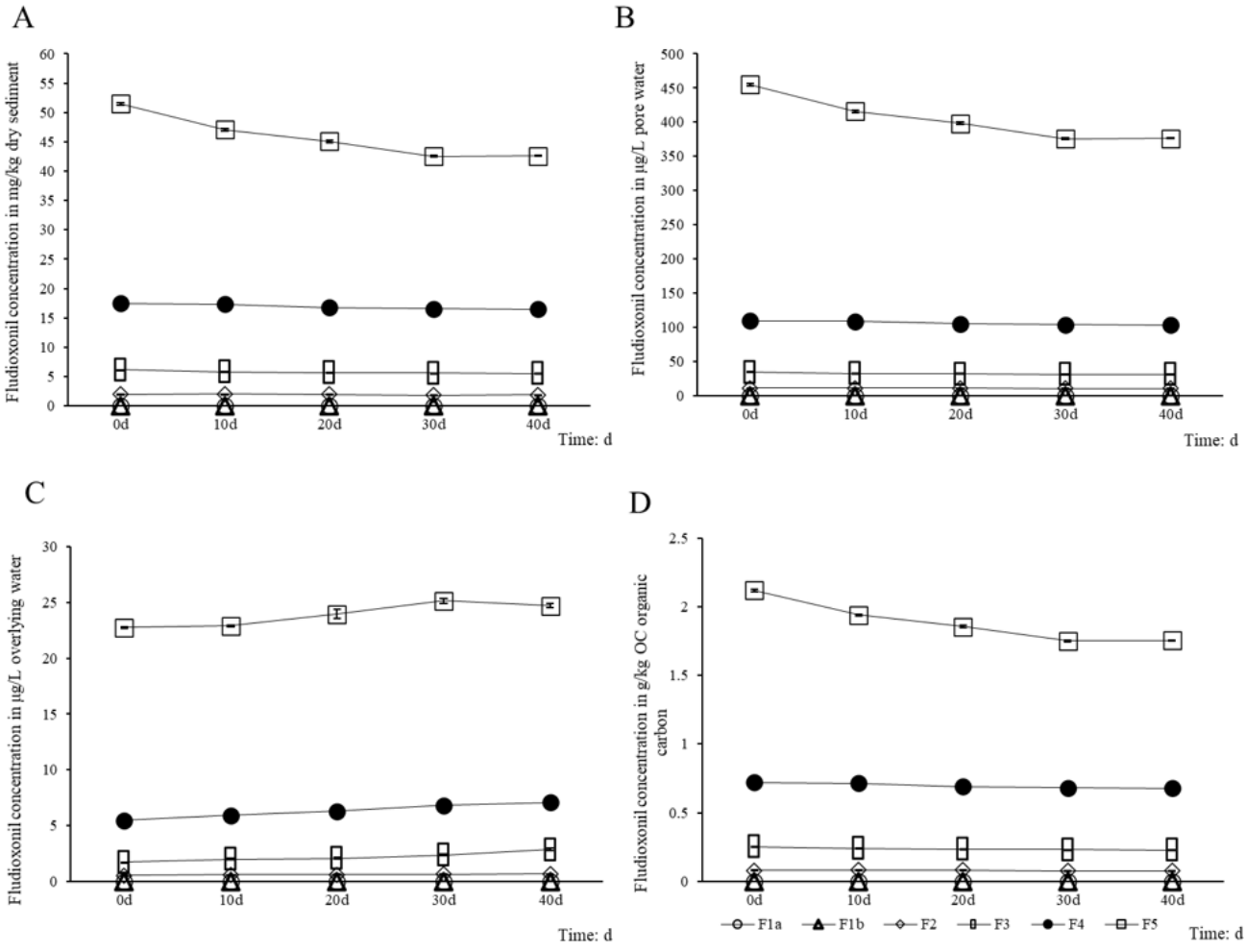

Figure 3

Dynamics of fludioxinil concentration in mg/kg dry sediment (panel A), μg/L pore water (panel B), μg/L overlying water (panel C) and g/kg organic carbon (panel D) in different treatments of the sediment-spiked indoor microcosm experiment.

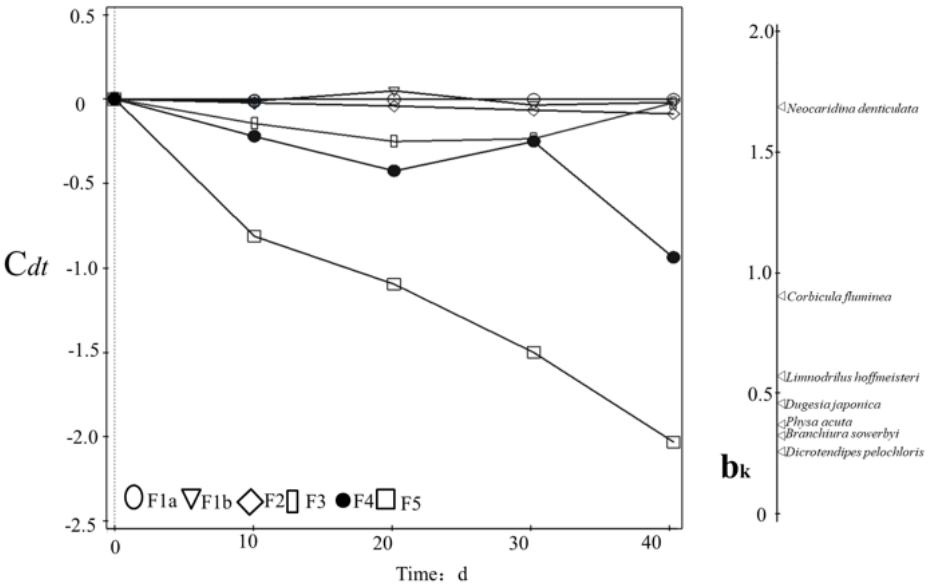

Figure 4

Principal response curve diagrams for the macroinvertebrate dataset in the total test system of indoor microcosms constructed with sediment that was spiked with different concentrations of fludioxonil. The x-axis indicate time. The Cdt canonical coefficients on the Y-axis indicate the difference between treatments

(F2-F5) and solvent controls (F1b). The bk species weights indicate the affinity of taxa with the PRC. The NOECs for the treatment-related responses are presented in Table 4.

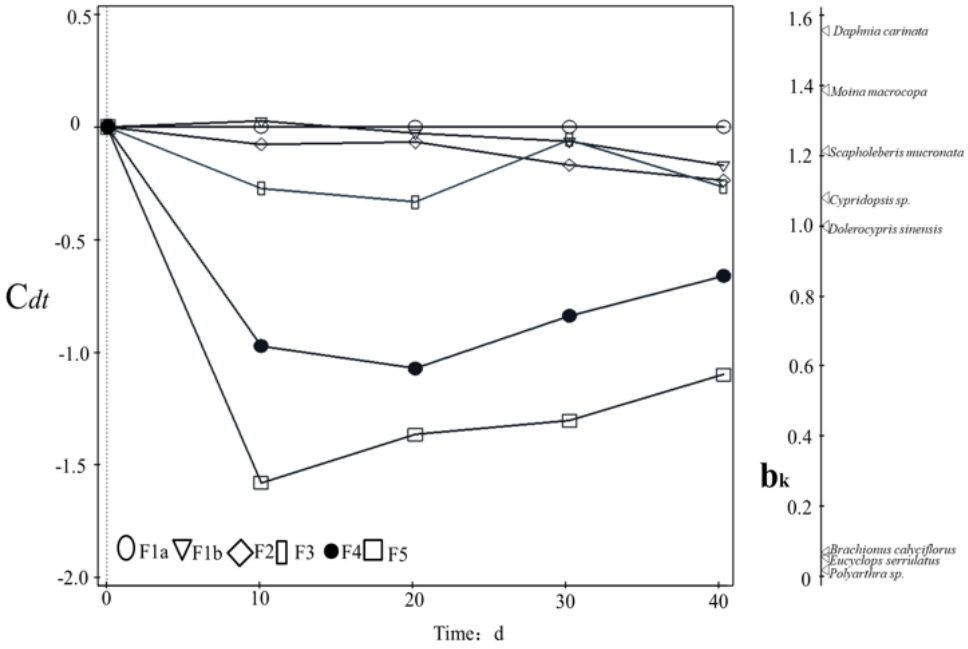

Figure 5

Principal response curve diagram for the zooplankton dataset of indoor microcosms constructed with sediment that was spiked with different concentrations of fludioxonil. The x-axis indicates time. The Cdt canonical coefficients on the Y-axis indicate the difference between treatments (F2–T5) and solvent controls (F1b). The bk species weights indicate the affinity of taxa with the PRC. The NOECs for the treatment-related responses are presented in Table 5.

Supplementary Files

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