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RESEARCH ARTICLE

Exposure pathways matter: Aquatic phototrophic communities respond differently to agricultural run-off exposed via sediment or water

Bastian H. Polst[1] | Joey Allen[2,3] | Franz Hölker[4,5] | Sabine Hilt[4] | Herwig Stibor[6] | Elisabeth M. Gross[3] | Mechthild Schmitt-Jansen[1]

[1]Department of Bioanalytical Ecotoxicology, Helmholtz-Centre for Environmental Research—UFZ, Leipzig, Germany; [2]Université de Rennes, CNRS, ECOBIO – UMR 6553, Rennes, France; [3]Université de Lorraine, CNRS, LIEC, Metz, France; [4]Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Berlin, Germany; [5]Institute of Biology, Freie Universität Berlin, Berlin, Germany and [6]Department of Biology II, Ludwig-Maximilians-University Munich, Martinsried, Germany

Correspondence
Bastian H. Polst
Email: bastian.polst@wur.nl

Mechthild Schmitt-Jansen
Email: mechthild.schmitt@ufz.de

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Abstract

1. Small shallow ponds are widespread but understudied water bodies in agricultural landscapes. Agricultural run-off (ARO) transports pesticides and nutrients into adjacent aquatic ecosystems where they occur dissolved in the water column or are bound to sediments. Consequently, aquatic communities are affected by ARO via different exposure pathways. We hypothesize that sediment-bound ARO mainly affects submerged rooted macrophytes, while phytoplankton and periphyton are more prone to ARO in water. These primary producers compete for resources resulting in a regime shift between alternative stable states of macrophyte or phytoplankton dominance. We hypothesize that warming increases nutrient release from sediments and thereby facilitates the occurrence of phytoplankton dominance.
2. Using a full-factorial microcosm design, we exposed aquatic primary producers to either sediment or water application of a mixture of common pesticides (terbuthylazine, pirimicarb, tebuconazole and copper) and nitrate at two concentrations and two temperatures (22°C and 26°C) for 4 weeks. Initial and final concentrations of pesticides and nitrate, final biomass of macrophytes, periphyton and phytoplankton, pesticide accumulation in macrophytes and changes in carbon, nitrogen and phosphorus content and selected exoenzyme activities in the sediment were measured.
3. We found lower final macrophyte biomass for both ARO treatments compared to controls, indicating a prevalence of negative effects by herbicides and competition for light with other phototrophs. In contrast, phytoplankton and periphyton biomass increased, but only when exposed to ARO via the water column, indicating a prevalence of positive effects by nutrient supply. Microbial carbon

and nutrient cycling in sediments was not affected by ARO. Higher temperature mitigated ARO-related effects on macrophytes under sediment exposure.

4. *Synthesis and application*. ARO poses a strong risk of submerged macrophyte loss and establishment of turbid conditions with phytoplankton dominance in aquatic ecosystems. In conclusion, exposure pathways as well as indirect and interacting effects of multiple stressors need to be considered when designing appropriate mitigation measures. Under climate change, we suggest to prioritize local measures as buffer strips a reduced use of pesticides and fertilizers, and sediment removal as appropriate measures to protect these vulnerable but widespread aquatic systems, which are highly relevant for biodiversity in agricultural landscapes.

KEYWORDS
agricultural run-off, exposure pathways, global warming, macrophyte, microalgae, multiple stressors, pesticides, regime shift, shallow lake

1 | INTRODUCTION

Multiple stressors threaten freshwater ecosystems worldwide and stressors often co-occur (Birk et al., 2020; Schinegger et al., 2012). In agricultural landscapes, pond ecosystems are mainly challenged by run-off of mixtures of nutrients and pesticides and understanding their interactive effects on ecosystem resilience is a key objective for pond ecosystem management (Hill et al., 2021).

Nutrients and pesticides transported by agricultural run-off (ARO) end up directly in the water column (Ulrich et al., 2013) or are rapidly transferred to sediments through absorption by sediment particles from the surface run-off or through subsurface flow (Niu et al., 2021). In both cases, aquatic sediments can act as a source (Abrantes et al., 2010; Machate et al., 2021) or sink (Chaumet et al., 2021; Mamta et al., 2019) of pesticides and nutrients. For instance, Machate et al. (2021) found more than 60 pesticide-related chemicals in sediments of shallow lakes in Northern Germany. Further, Mahler et al. (2020) found 25 pesticides in streambed sediments and even more pesticides at higher concentration in periphyton. Organisms living in close contact with sediments such as rooted macrophytes and microbial communities at the surface of the sediments and within the sediments could thus experience higher exposure to pesticides compared to exposure via the water phase. These organism groups could even influence the translocation from sediment to water (Diepens et al., 2014; Mahler et al., 2020). This type of exposure can affect macrophyte communities negatively, while the phytoplankton community remains unaffected (Machate et al., 2021).

Agriculturally used landscapes are characterized by abundant small, shallow lakes or ponds. However, even though shallow lakes and ponds are among the most biodiverse and ecologically important freshwater habitats, globally, they are generally underrepresented in research and monitoring. For example, they are not considered in the EU-water framework directive (Hill et al., 2021; Verpoorter et al., 2014). Highest biodiversity in shallow lakes and ponds as well as their surrounding landscape is supported by a dominance of macrophytes but eutrophication can lead to regime shifts to phytoplankton dominance with significant consequences for ecosystem functioning (Hilt et al., 2017; Law et al., 2019; Scheffer et al., 1993). High nutrient concentrations in the water phase in combination with pesticides have been shown to promote regime shifts from macrophyte to phytoplankton dominance in model ecosystems, mimicking small, shallow water bodies (Polst et al., 2022; Vijayaraj, Kipferler, et al., 2022). Whether the interactive effects of these stressors are maintained during sediment exposure is unknown, but of importance for the management macrophyte dominated shallow lakes and ponds.

Pesticides and nutrients present in sediments have been recognized to influence not only rooted macrophytes but also the microbial communities inhabiting these sediments (Xie et al., 2016). The crucial role of microbial activities in nutrient cycling suggests that any impairment in the sediment microbial community may have far-reaching effects on nutrient availability for rooted macrophytes. Additionally, within the benthic boundary layer, microalgae known as epipsammon, may play a critical role in the exchange of nutrients and pesticides between the sediment and the water column, thereby affecting the occurrence of regime shifts between primary producers in the water column.

Elevated water temperatures lower the threshold for ARO-induced regime shifts when ARO is applied via the water phase (Polst et al., 2022). It is unclear, however, whether the same holds true when ARO is exposed resp. released via the sediment. Nutrient release from the sediment likely increases with higher temperatures (Duan & Kaushal, 2013; Shinohara et al., 2021). The impact of elevated temperatures on the release of pesticides from the sediment is yet unclear. Understanding the interactions of both stressors and both exposure pathways is crucial for the success of future management activities like defining critical thresholds of agricultural run-off to aquatic systems (Cuenca-Cambronero et al., 2023; Liu et al., 2015). Next to the release of nutrients and pesticides from the sediment to the water column, many related processes in the benthic boundary layer may be influenced by elevated temperatures, for example microbial degradation or accumulation of pesticides by organisms. At the same time, this may be counteracted by an increased metabolism and therefore higher growth rates of phototrophic organisms. Whether higher temperature leads to a higher risk of regime shifts facilitated by ARO associated to the sediment is still an open question.

To answer these questions we tested three hypotheses: (1) Exposure to ARO through the sediment reduces the risk of shifts toward a turbid phytoplankton-dominated state compared to exposure via the water column. (2) In contrast, pesticides in the ARO mixture could negatively affect microbial activities in sediments. (3) Further, warming will lead to a higher remobilization of ARO from the sediment and thereby diminish the differences between exposure pathways, leading to regime shifts via both exposure pathways.

2 | MATERIALS AND METHODS

2.1 | Microcosm setup

Microcosms were constructed as described in Polst et al. (2022). In brief, cylindrical glass vases (height 40 cm, diameter 25 cm) served as microcosms and were filled with 8 L Volvic® mineral water (Danone Waters Deutschland GmbH) (Figure 1). Sediment was mixed according to the OECD protocol *Test No. 239: Water-Sediment Myriophyllum Spicatum Toxicity Test* (OECD, 2014) with additional 1% nettle powder. Three macrophyte species typical for temperate shallow aquatic systems (Hilt & Gross, 2008), *Myriophyllum spicatum*, *Potamogeton perfoliatus* and *Elodea nuttallii*, were collected from presumably unimpacted sites (no permission required) and transferred to the laboratory. They were cut to 8-cm stems, planted into sediment and kept in Volvic water with the aim to initiate root growth. After 1 week, two stems of *M. spicatum* and *E. nuttallii* as well as one stem of *P. perfoliatus* were planted into glass bowls (height 8 cm, Ø 15 cm) filled with ~600 g sediment covered by an upper layer of sand (1 cm). The glass bowls with the sediment and planted macrophytes were then placed in the microcosm. Glass slides (3 cm × 25 cm) were placed vertically on top of the sediment providing a surface for periphyton growth representing periphyton on macrophyte leaves. Natural phytoplankton ($1 \times 10^6\,\mu m^3\,mL^{-1}$ biovolume per microcosm) and periphyton ($2\,cm^2$ per microcosm) communities from nearby presumably unimpacted shallow ponds were used as microalgae inoculum. Temperature control was established via air conditioning in the culture room and microcosms in the treatments with elevated temperature were placed on heating mats (40 W). Light intensity at the water surface was approximately $77\,\mu mol\,s^{-1}\,m^{-2}$ (see Polst et al., 2022).

2.2 | Study design

An artificial mixture representing pesticides commonly found in agriculturally impacted aquatic ecosystems (Halbach et al., 2021; Lefrancq et al., 2017; Wijewardene et al., 2021) had been selected for our study. Its ingredients represent each major group of pesticides (herbicide, insecticide, fungicide) plus copper as inorganic fungicide and a high nitrate concentration. The latter was chosen due to its increasing role in agriculturally impacted streams (James et al., 2005; Xu et al., 2014). Several concentrations of this ARO mixture had been tested for triggering regime shifts by Allen et al. (2021), Vijayaraj, Kipferler, et al. (2022) and Polst et al. (2022). ARO concentrations for this study were chosen based on these former results. A three-factorial approach was used to account for two ARO concentration levels (1 & 4-fold), two exposure pathways (water & sediment) and two temperatures (ambient ≈ 22°C & heated ≈ 26°C) (Table 1). In a control treatment, a solvent was used for the pesticides (dimethylsulfoxid) to guarantee exact dosing and was applied to both, the sediment and the water phase of the control treatment microcosm without any ARO compound. The ARO and the solvent control were applied as single pulse marking the start of the

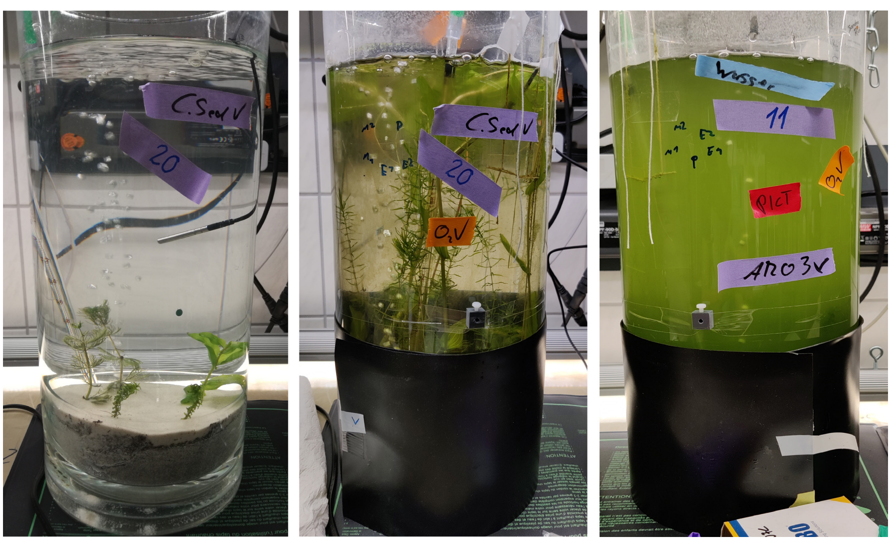

FIGURE 1 Microcosms at the start of the experiment (left), warmed (26°C) control treatment at the end of the experiment with a dominance of submerged macrophytes (middle) and warmed (26°C) treatment with highest concentration of agricultural runoff (ARO 4) developing phytoplankton dominance (right).

TABLE 1 Overview on nominal concentrations of compounds in the water column included in the agricultural run-off mixture (ARO), their stressor group, $\log K_{OW}$ (Tomlin, 2004).

	N-NO3	Terbuthylazine	Pirimicarb	Tebuconazole	Copper
Stressor	Nutrient	Herbicide	Insecticide	Fungicide	Fungicide
$\log K_{OW}$	n.a.	3.4	1.7	3.7	n.a.
ARO 1	2250 $\mu g\,L^{-1}$	0.75 $\mu g\,L^{-1}$	3.75 $\mu g\,L^{-1}$	22.5 $\mu g\,L^{-1}$	10.5 $\mu g\,L^{-1}$
ARO 4	9000 $\mu g\,L^{-1}$	3 $\mu g\,L^{-1}$	15 $\mu g\,L^{-1}$	90 $\mu g\,L^{-1}$	42 $\mu g\,L^{-1}$

experiment. The elevated temperature of Δ+4°C was selected based on Woolway et al. (2021), who predicts such a rise during heatwaves in lakes. Three times per week nutrients (nitrate and phosphate) were added according to Vijayaraj, Kipferler, et al. (2022) to prevent nutrient limitation within the microcosms.

2.3 | Sampling

Available light and water temperatures were logged on top of the sediment every 5 min (Hobo Pendant data logger; Onset Computer Cooperation, USA) and light sensors were cleaned weekly to avoid shading by attached algae. Throughout the experiment, water samples were taken bi-weekly for organic pesticide analysis and weekly for nutrient analysis. Samples of the sediment porewater were taken at the start (representative replicates) and the end of the experiment to avoid distribution of the microcosms. Samples for analysis of copper concentrations in the water were taken at the start and at the end of the experiment, copper concentrations could not be analysed in the sediment porewater due to the low extracted volume. To monitor the phytoplankton development over the course of the experiment, weekly phytoplankton samples (dry weight) were taken by filtration (0.7 μm glass-fibre filters; Labsolute, Germany). Four weeks after start of ARO exposure, macrophytes, phytoplankton, periphyton from glass slides and the periphytic community that developed at the surface of the sediment (epipsammon) were sampled for biomass acquisition. Three sediment cores were collected using a 2 cm diameter syringe with the tip cut off. From these three cores, the bottom layer (mixed sediment) was pooled for elemental C, N, P analysis and measurement of microbial activity. Subsamples were frozen at −20°C as this treatment was shown to preserve enzymatic activity (Flores et al., 2012). The top layer (approx. 1 cm) was mixed for pigment analysis of epipsammon. Macrophytes were carefully removed from the sediment and root and stem length were measured. The apical 10 cm of *M. spicatum* were sampled separately and frozen in liquid nitrogen for pesticide analysis. The residual shoots were dried at 55°C for 24 h to derive the respective dry weight. Periphyton was scratched off the glass slides, filtered (0.7 μm), weighted for dry weight (DW) measurement.

2.4 | Nutrient and pesticide analysis

Water samples for pesticide and nutrient analysis were filtered (0.22 μm, cellulose acetate filter). Samples of sediment pore-water were obtained through sedimentation of the sediment slurry and measuring pesticides in the supernatant. The three organic pesticides in samples from the water column and the supernatant sediment pore-water samples were analysed using an LTQ-OrbiTrap (Thermo Scientific, USA) (Finckh et al., 2022). Copper concentration in the water was analysed according to Vijayaraj, Kipferler, et al. (2022). Dissolved inorganic nutrients (PO_4^{3-}, NO_3^-) were analysed photometrically according to DIN-EN-26777 (1993), DIN-EN-ISO 13395 (1996) and DIN-EN-ISO-6878 (2004). Copper and dissolved inorganic nutrients were not measured in the sediment porewater as the retrieved volume was too low. Elemental C and N concentrations of dried sediments were determined at the end of the experiment with a CHN elemental analyser (Carlo Erba, NA 2100; Thermo Quest CE International). Total P of sediment samples was oxidized to orthophosphate using alkaline persulfate and then quantified by ammonium molybdate spectrophotometric method. To determine the pesticide concentrations in *M. spicatum*, the upper 10 cm were freeze-dried and pesticides were extracted following a QuEChERS protocol (Desiante et al., 2021). Extracts were measured using an LC-HR MS based on the method in Finckh et al. (2022) and the retrieved pesticides were quantified based on extracted biomass.

2.5 | Photosynthetic pigment analysis

For epipsammic community pigment analysis, the collected upper sediment layer was freeze dried in the dark for 24 h before addition of the extraction solvent (buffered methanol with 1 M ammonium acetate). Extraction solvent volume was adjusted in order to have approximately 1.5 mL of solvent above the sand (between 9 and 11 mL were used). Samples were then sonicated for 3 min in an ultrasound bath containing water and ice to limit warming of the samples, incubated overnight at −20°C, and sonicated again at same conditions. After centrifugation (10,000 g, 5 min at 2°C), 1 mL of supernatant was collected. The collected extracts were analysed as described in Capdeville et al. (2019).

2.6 | Exoenzymatic activities

For enzymatic activity of aminopeptidase, ß-glucosidase and phosphatase measurements, 0.5 g of sediment from each microcosm was collected and kept at −20°C until enzyme extraction (Flores et al., 2012). For enzyme extraction, the sediment was mixed with

5 mL of extraction buffer (pH 7 phosphate buffer with 22.2 g L^{-1} $CaCl_2$ and 20 g L^{-1} poly-vinylpyrrolidone, 0.5 mL L^{-1} Tween 80) for 1 h. After centrifugation, the supernatant was collected. Supernatant subsamples were placed for 3 h in boiling water to inactivate the enzymes as negative controls. The enzymatic activity was tested using three different substrates: L-Leucine-7-amido-4-methylcoumarin for leucine-aminopeptidase activity, 4-methylumbelliferone-glucopyranoside for β-glucosidase activity and 4-methylumbelliferone-phosphate (Sigma-Aldrich, USA) for phosphatase activity. Extracts and inactivated extracts were placed in 96-well plates (38 μL per well, with 4 technical replicates) with 250 μL of 200 μM enzyme substrate per well. Fluorescence was measured at 455 nm for β-glucosidase and phosphatase activity and 445 nm for aminopeptidase activity with an excitation wavelength at 365 nm after 8 h of exposure using a SAFAS Xenius fluorimeter (SAFAS, Monaco).

2.7 | Statistics

Statistical assessment was done using R (R Core Team, 2020). Effect sizes (Hedges' *g*) and their confidence interval were calculated using the *esc* package (Lüdecke, 2019). Effect sizes were calculated for each temperature separately with the respective controls as reference treatment. Effects are considered significant when the effect size confidence interval does not overlap with zero. We classified effects as regime shifts, when macrophytes showed a significant biomass decline parallel to a significant increase in phytoplankton biomass.

3 | RESULTS

3.1 | Physico-chemical parameters

Over the course of the experiment, the pH increased from 7.9 up to 9.7 and conductivity ranged from 240 to 358 μS cm^{-1}, both peaking in water-exposed treatments with higher ARO concentration. Water temperatures differed by 3°C at the start of the experiment, with approximately 21°C for the unheated and 24°C for the heated treatments. Throughout the experiment, both temperatures increased slightly: while at the last day before the final sampling, the unheated microcosm averaged approx. 23°C the heated ones averaged approx. 25.5°C. Light availability at the bottom of the microcosm decreased throughout the experiment, with minima occurring in treatments with highest ARO exposed via the water (Appendix SF1).

3.2 | Concentrations of the agricultural run-off mixture

3.2.1 | Nutrient and pesticide concentrations in the water phase and in sediment porewater

Nitrate and organic pesticide concentrations in the water phase at the beginning of the experiment were within ±10% of their nominal values of the respective ARO treatment (Table 2). Initial organic pesticide concentrations in the porewater of sediment-exposed

TABLE 2 Start (Day 0) concentrations of agricultural run-off (ARO, mean ± SD, $N\text{-}NO_3$, terbuthylazine, pirimicarb, tebuconazole, copper) in the water phase and in the sediment porewater of the treatments with ARO exposure via the sediment one day after start of the exposure (<dl: below detection limit, na: not available, C: control, W: water-exposed, S: sediment-exposed, 1: lowest concentration tested (ARO 1), 4: highest concentration tested (ARO 4)).

		$N\text{-}NO_3$ mg L^{-1}	Terbuthylazine μg L^{-1}	Pirimicarb μg L^{-1}	Tebuconazole μg L^{-1}	Copper μg L^{-1}
Ambient						
C	Water phase	1.3 ± 0.3	<dl	<dl	<dl	0.6 ± 0.3
W 1	Water phase	3.4 ± 0.3	0.7 ± 0.0	3.5 ± 0.03	17.9 ± 1.2	4.7 ± 0.4
W 4	Water phase	10.3 ± 0.4	3.1 ± 0.8	16.1 ± 4.4	77.2 ± 15.1	18.9 ± 2.5
S 1	Porewater	na	2.1 ± 0.2	40.1 ± 3.5	15.2 ± 2.5	na
	Water phase	1.3 ± 0.3	<dl	<dl	<dl	0.6 ± 0.1
S 4	Porewater	na	8.3 ± 0.8	155.1 ± 14.5	59.9 ± 9.0	na
	Water phase	2.5 ± 0.2	<dl	<dl	<dl	0.7 ± 0.2
Heated						
C	Water phase	1.4 ± 0.1	<dl	<dl	<dl	0.6 ± 0.2
W 1	Water phase	3.5 ± 0.1	0.85 ± 0.1	4.1 ± 0.4	23.6 ± 3.8	5.2 ± 0.4
W 4	Water phase	10.3 ± 0.3	2.80 ± 0.0	14.5 ± 0.3	72.0 ± 1.7	18.7 ± 3.1
S 1	Porewater	na	2.1 ± 0.2	40.1 ± 3.5	15.2 ± 2.5	na
	Water phase	1.4 ± 0.1	<dl	<dl	<dl	0.4 ± 0.1
S 4	Porewater	na	8.3 ± 0.8	155.1 ± 14.5	59.9 ± 9.0	na
	Water phase	2.5 ± 0.1	<dl	<dl	<dl	0.8 ± 0.4

treatments varied heavily between pesticides and did not represent the original ratios of the ARO mixture, anymore. The sediment-porewater concentration of terbuthylazine was approximately 2.5 times higher than in the water column of the respective water-exposed treatments, pirimicarb was approximately 9.5 times higher, and tebuconazole was approximately four times higher. Yet, the concentrations in the water column of sediment-exposed treatments were below detection limit. Copper concentrations in the water-exposed treatments were significantly lower than anticipated (Table 2). Phosphate could be measured at a low concentration across all treatments at the start of the experiment but concentrations decreased below the detection limit later in the experiment (Appendix SF3).

In the sediment-exposed treatments low amounts of nitrate translocated from the sediment to the water phase in the first week of the experiment (Appendix SF2). During the rest of the experiment, overall nitrate concentrations decreased and were mostly close to, or below detection limit. In parallel, pirimicarb translocated from the sediment to the water. In the water-exposed treatments, pesticide concentrations in the water phase decreased with time (Table 2; Figure 2).

3.2.2 | Organic pesticide concentrations in *Myriophyllum spicatum*

All pesticides were found in the apical part of *M. spicatum* but showed different accumulation depending on the substance (Figure 3): terbuthylazine and tebuconazole occurred at significantly higher concentrations in *M. spicatum* grown in treatments with ARO exposed to water than in treatments with ARO exposed to sediments. Exposure pathways did not lead to differences between treatments for pirimicarb.

3.3 | Development of photoautotrophic compartments

In treatments with lower ARO concentrations at ambient temperatures, macrophyte biomass was significantly lower in the water-exposed treatment, while no significant effect was found in the sediment-exposed treatment (Figure 4). When exposed to higher ARO concentrations, both exposure pathways showed negative effects for macrophyte biomass. Phytoplankton showed a negative

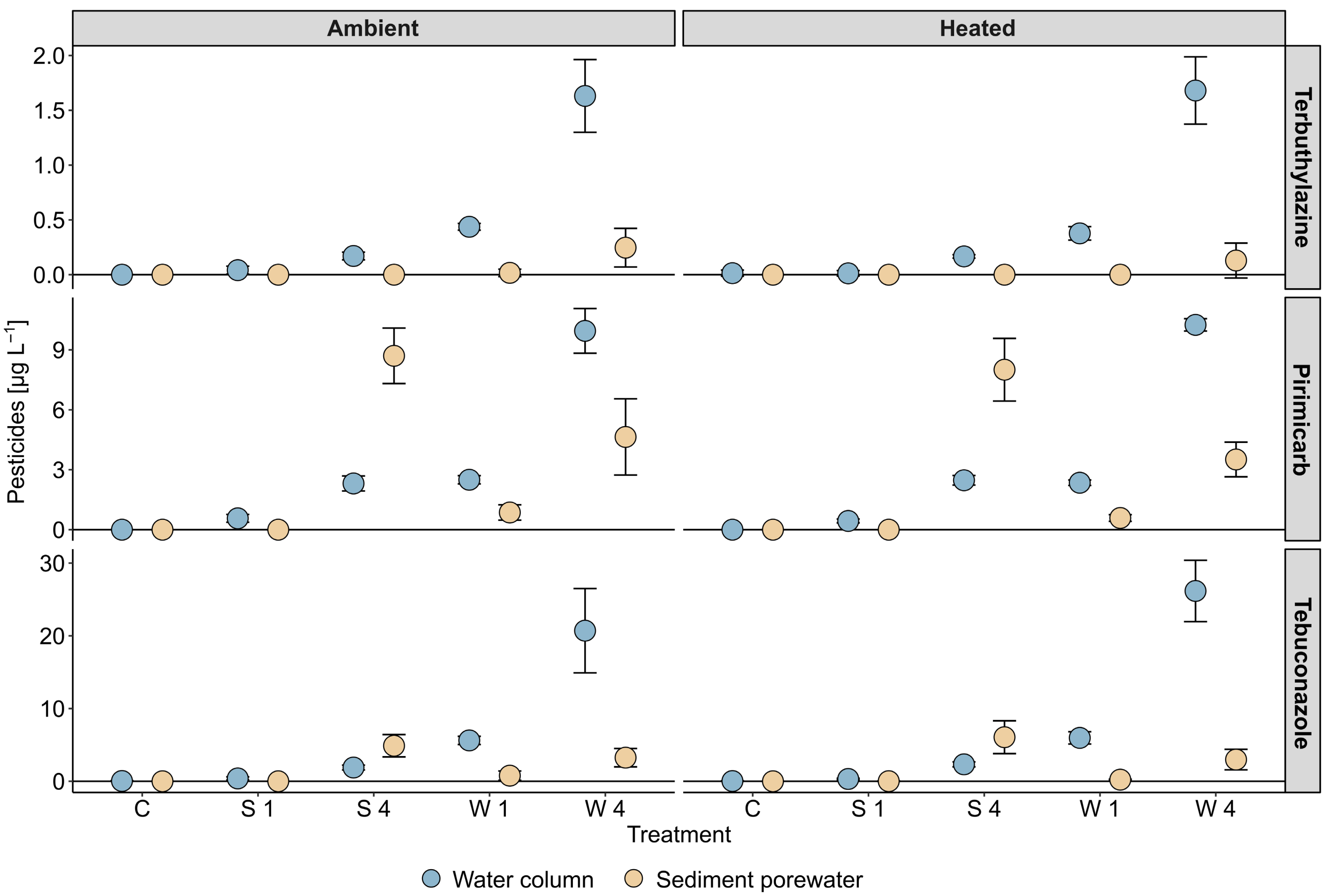

FIGURE 2 Organic pesticide concentrations at the end of the experiment measured in the water column (blue) and sediment porewater (brown) (S 1: low concentrations sediment exposed ARO, W 1: low concentrations water exposed ARO, S 4: high concentrations sediment exposed ARO, W 4: high concentrations water exposed ARO).

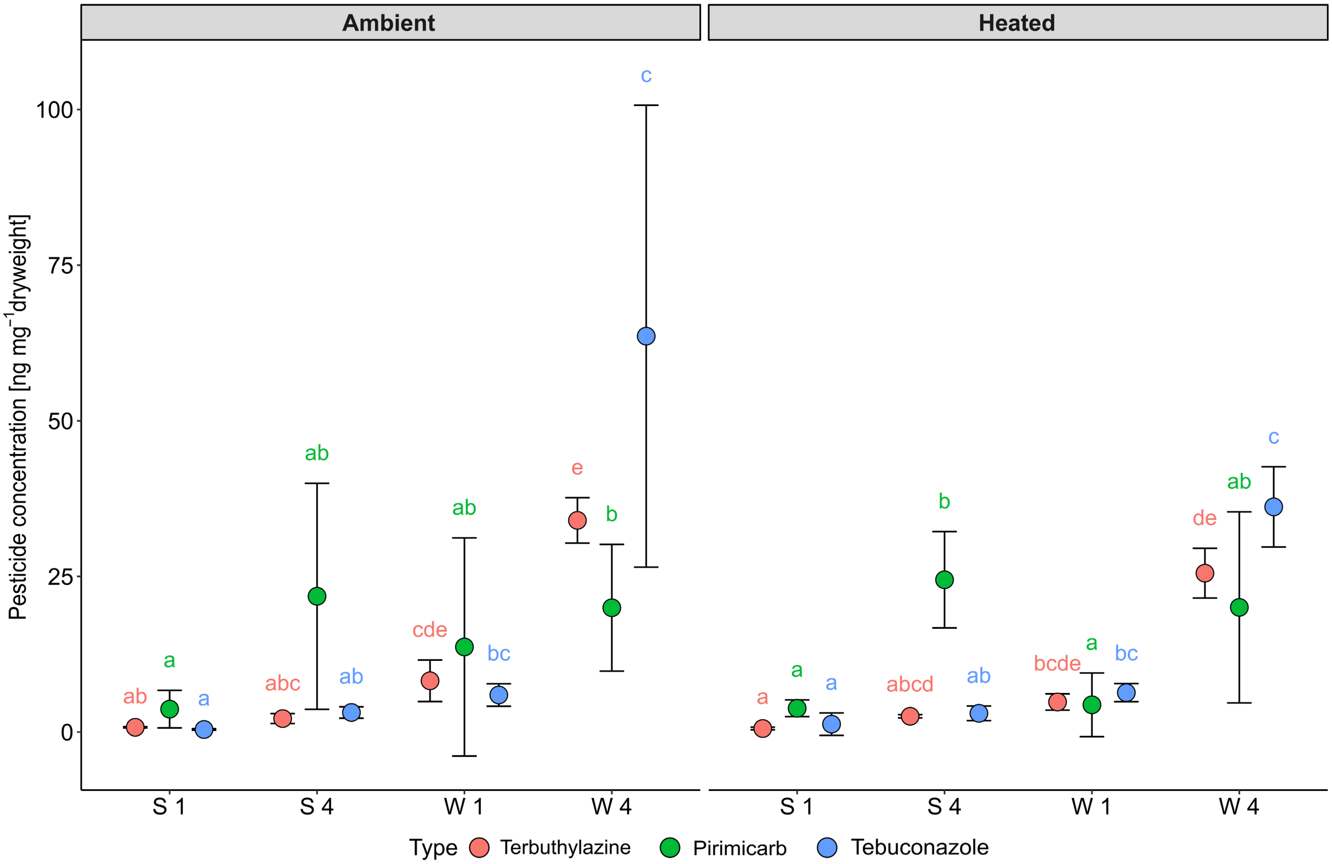

FIGURE 3 Organic pesticide concentrations (red = terbuthylazine, green = pirimicarb, blue = tebuconazole) in the apical 10 cm of *Myriophyllum spicatum* at the end of the experiment. Plant biomass from the control treatments were used to test the recovery of pesticides of the extraction method and are therefore not available, but control microcosms were previously found to be unimpacted by pesticides. Letter coding based on Dunn's post-hoc test ($p < 0.05$) and applies to the three pesticides separately as indicated by the respective colours. Treatments: S 1 = sediment exposed ARO 1, W 1 = water exposed ARO 1, S 4 = sediment exposed ARO 4, W 4 = water exposed ARO 4.

effect in the first week for the water-exposed treatment but both treatments showed positive effects in the later weeks of the experiment. Additionally, epipsammon showed positive responses in the water-exposure treatments. No further effects on phototrophic biomass were observed in the sediment-exposed scenario. In the heated treatments, only epipsammon showed a negative response to the lower ARO concentration, otherwise no effects were observed. For the higher ARO concentration under warming, macrophyte biomass decreased in the water-exposed treatment significantly in comparison to control as well as in comparison to the sediment-exposed treatment. Phytoplankton biomass decreased initially in the water-exposed treatment but showed an increase at the end of the experiment. A regime shift toward phytoplankton dominance was indicated by the decline in macrophyte biomass parallel to an increase in phytoplankton biomass in both ARO treatments at ambient temperatures (22°C) and the higher ARO treatment at warmed conditions (26°C).

The effects in the water-exposed treatments were similarly driven by the response of all three tested macrophyte species, while in the sediment-exposed treatments only *P. perfoliatus* showed a significant decline in dry weight (Appendix SF5). The root: shoot (length) ratio of all three macrophyte species did not show significant changes for any treatment (data not shown).

3.4 | Microbial functions and nutrients in sediments

Exposure pathways led to only few differences between treatments in elemental content of macroelements and exoenzymatic activity in the sediment layer (Figure 5). While carbon and nitrogen content in sediments showed no exposure-related effect, the phosphate content was significantly higher at the highest ARO concentration in the sediment-exposed treatments than in the other treatments.

4 | DISCUSSION

Our results demonstrate that exposure pathways of common chemical stressors in agricultural landscapes matter for their effects on primary producers and sediment-nutrient dynamics in shallow

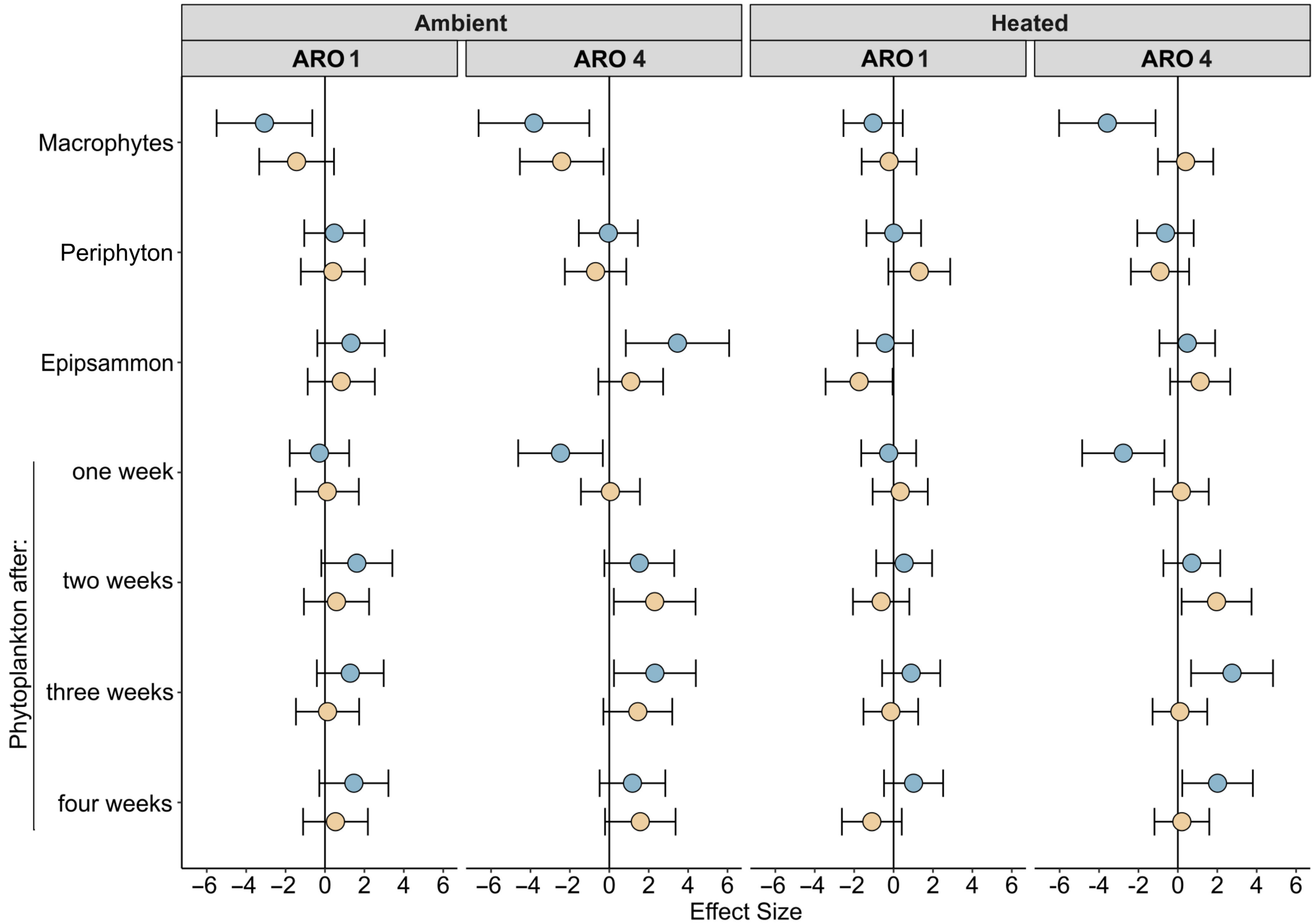

FIGURE 4 Effect sizes (mean ± SD; blue dots = exposure of agricultural run-off (ARO) via water column, brown dots = ARO exposure via sediment) for phototrophic biomass (macrophyte, periphyton and episammon at the end of the experiment and phytoplankton over the course of the 4 weeks after exposure).

lakes and ponds. Knowledge of the interacting effects of multiple stressors is a key issue for conservation and management (Brown et al., 2013; Côté et al., 2016; Hill et al., 2021). Consequently, mitigation and restoration measures must be designed accordingly to restore and maintain the highest ecological quality and associated ecosystem services. These measures must not only consider the indirect effects of multiple agricultural stressors, but beyond the role of different exposure pathways including the translocation of pesticides.

4.1 | Fate of pesticides

When ARO was added to the sediments, the three organic pesticides partly leached into the water column toward the end of the experiment; thus, the sediment acted as a source of pollution for the water phase. The concentrations of pesticides in the sediment pore water of the ARO mixture decreased at a higher rate compared to those in the water column. This suggests that the sediment plays a crucial role as a source of pesticides in dependence of their physico-chemical properties (log $K_{OW,}$ see Table 1). This is especially important as sediments might store pesticides over long timescales and release them back to the water phase over time. Pesticides not recently applied in the pond catchments or even banned for longer times, are regularly found in pond water indicating the relevance of the sediment as a source of pesticides (Chaumet et al., 2021; Rasmussen et al., 2015). This finding highlights the importance of addressing sediment contamination as part of restoration strategies aimed at mitigating the effects of ARO.

Moreover, we found that the three organic pesticides were detected in the apical shoots of *M. spicatum*, indicating their uptake from both the water column and the sediment porewater. Uptake could derive from different pathways: uptake directly from the water column or uptake from sediment pore-water via roots and transfer to aboveground parts of the macrophytes. This underscores the importance of considering the potential uptake of contaminants by aquatic plants when developing restoration strategies. The translocation of pollutants from the sediment pore water to the water column, followed by uptake by macrophytes, emerged as a primary pathway (Diepens et al., 2014). However, translocation and uptake pathway depend on hydrophobicity of the pesticides. The

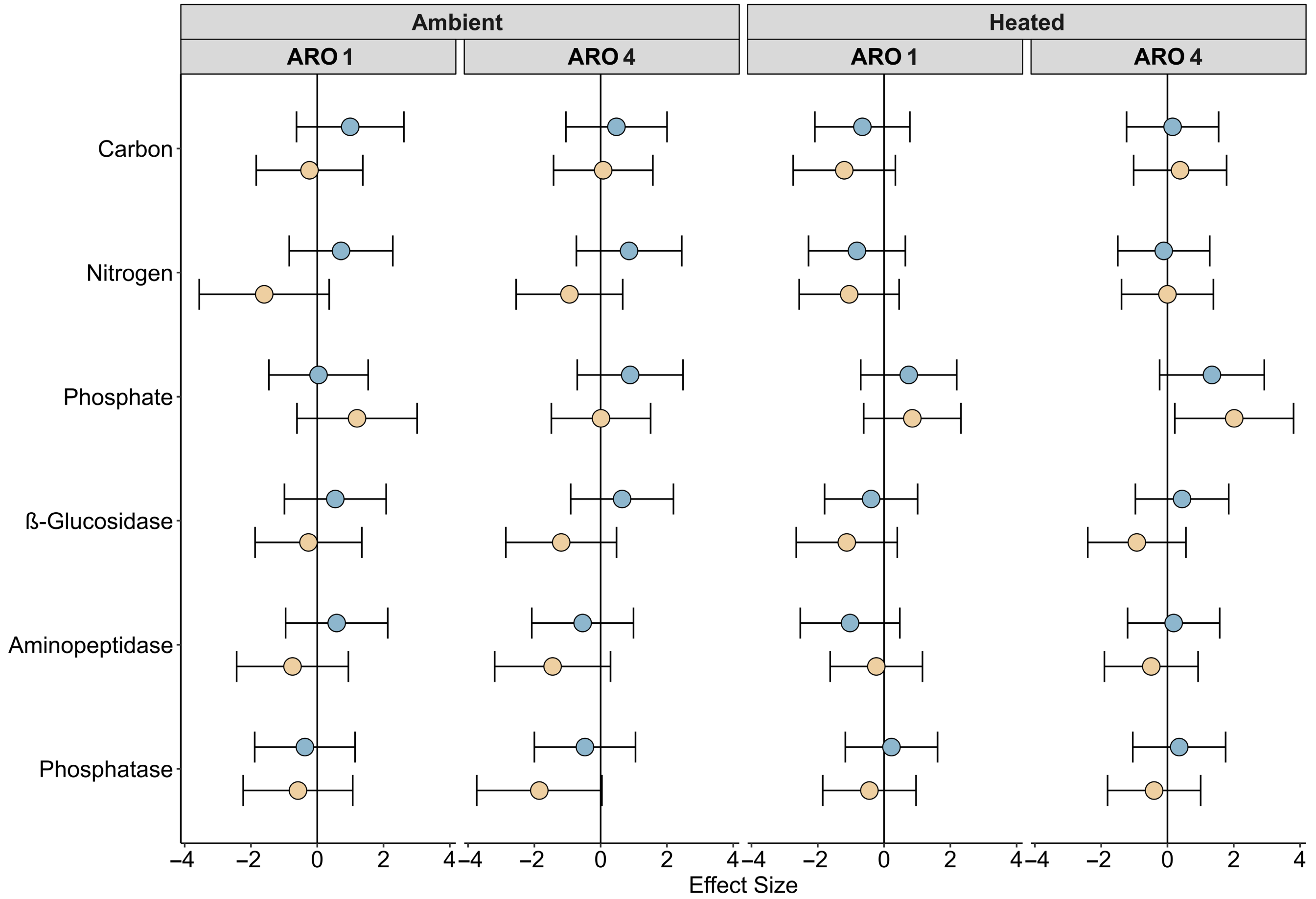

FIGURE 5 Effect sizes (mean ± SD; blue dots = exposure of agricultural run-off (ARO) via water column, brown dots = ARO exposure via sediment) for the elemental composition of the sediment (content in carbon, nitrogen and phosphorus) and exoenzymatic activity (ß-glucosidase, aminopeptidase, phosphatase).

low internal concentrations of the herbicide terbuthylazine likely contributed to the lack of effects on *M. spicatum* in the sediment-exposed treatments. This knowledge can guide restoration efforts by emphasizing the role of macrophytes in pollutant removal and the potential for using them in phytoremediation approaches.

Regarding copper pollution, our study revealed that complexation processes resulted in decreased copper concentrations. Although the toxic effects of copper were likely lower than anticipated in our study, its persistence and accumulation potential pose significant risks in the field, particularly in the context of organic agricultural practices. Therefore, effective preventive measures should consider not only organic pesticide use but also the potential for copper pollution. In conclusion, our study emphasizes the importance of considering the applied ecological aspects of sediment pollution and restoration in the context of agricultural run-off. Understanding the fate and effects of pollutants in sediments and water, as well as their interactions with macrophytes, is crucial for developing targeted restoration strategies and preventive measures. By integrating this knowledge into restoration and pollution prevention practices, we can make significant strides toward preserving and rehabilitating aquatic ecosystems impacted by agricultural run-off.

4.2 | Nutrient dynamics

Surprisingly, the addition of ARO to the sediment or water did not affect the N-content of the sediment, despite the presence of nitrate in the tested ARO mixture. This suggests that nitrate underwent rapid leaching from the sediment into the water column or a high denitrification rate in the sediment exposed treatments. This contributed to the lack of N-induced effects in the sediment and even led to a short increase in phytoplankton biomass early in the experiment. Overall, nutrient concentrations in the water in both exposure pathways decreased rapidly and were probably converted into biomass by either phytoplankton or benthic microalgae (see Appendices SF3 and SF4).

Interestingly, our results revealed that ARO exposure did not negatively affect nutrient cycling related to microbial activities in the sediment, thereby not confirming our second hypothesis. Additionally, contrary to our third hypothesis, elevated temperatures did not lead to a higher resuspension of ARO from the sediment into the water phase. These findings highlight the complex interactions between ARO, sediment and temperature, indicating that multiple factors influence the fate and effect of pollutants in aquatic systems.

Despite higher nitrate loading, no significant increase in epipsammic biomass with sediment exposure to ARO was observed, potentially due to herbicide effects. Higher nitrate levels in sediments indicate a negative effect on phosphorus uptake by macrophytes or a lower release to the water column. In conclusion, a holistic picture of multiple stressor interactions needs to be considered to choose appropriate mitigation measures and contrasting effects of agricultural stressors, for example by fertilizers and pesticides need to be taken into account.

4.3 | Exposure related regime shifts

Lower macrophyte growth and an increased phytoplankton or periphyton biomass, indicative for a potential regime shift, were only observed in the water-exposed treatments. This aligns with findings from other microcosm studies testing the same ARO mixture (Allen et al., 2021; Polst et al., 2022; Vijayaraj, Laviale, et al., 2022), emphasizing the ecological interactions following ARO exposure. It is important to note that the decline in macrophyte biomass was not observed for all macrophyte species, highlighting the role of dominant species in the field (Cedergreen et al., 2004; Wu et al., 2021).

The observed decrease of macrophyte biomass can be caused directly by toxicity of the pesticides but also indirectly by shading by a nitrate-induced phytoplankton bloom. An interaction of both effects was found by Wendt-Rasch et al. (2004) and Polst et al. (2022) in microcosm experiments including *M. spicatum*. Although the pesticide concentrations used in our study were relatively low compared to EC_{50} values for macrophytes (Giddings et al., 2013), previous studies using similar pesticide concentrations did not identify clear toxic effects on macrophytes (Allen et al., 2021; Vijayaraj, Laviale, et al., 2022). Consequently, the decline in macrophyte biomass was likely caused by the higher phytoplankton density, which reduced light availability or an interaction with pesticides rather than direct pesticide exposure.

Interestingly, exposure to ARO via the sediment only affected macrophyte growth and had no impact on microalgae, supporting the findings of Machate et al. (2021). Pesticides present in the sediment porewater can negatively affect macrophyte growth through direct effects unrelated to shading by microalgae. Again, it is important to consider species-specific sensitives toward pesticides as two of the three macrophyte species were not affected in their growth after 4 weeks of ARO exposure via the sediment.

During sediment exposure, elevated temperatures negated the previously discussed negative effects of ARO on macrophytes, contrasting with the heated water-exposed treatments. The convergence of effects predicted by our third hypothesis, resulting from increased ARO remobilization from the sediment, was not observed. Apparently, the negative effect of pesticides originating from the sediment are counteracted by an increased metabolism and growth of macrophytes, which has also been described by Vijayaraj, Kipferler, et al. (2022). Nevertheless, longer exposure of ARO via the sediment than in our study, which converges with more realistic field scenarios, may eventually result in a loss of macrophytes.

These findings highlight the complex interactions between ARO, macrophytes, phytoplankton, and sediment dynamics in aquatic ecosystems. Understanding the interacting effects of ARO is crucial for developing effective restoration and management strategies to mitigate the potential regime shifts caused by agricultural run-off. This is of special importance, when antagonistic effects derive from global warming, a stressor which cannot be managed, locally (Brown et al., 2013). Therefore, management activities of local stressors need to be adjusted under climate change, according to these interaction patterns.

5 | CONCLUSIONS

Macrophyte-dominated small and shallow aquatic ecosystems fulfil important functions in agricultural landscapes, especially as a habitat for a diverse flora and fauna. Exposure to high nutrient and pesticide loadings via water induce shifts to the less diverse, phytoplankton-dominated state (Allen et al., 2021; Polst et al., 2022; Vijayaraj, Kipferler, et al., 2022). A prevalence of effects by ARO in the water phase, as shown in our study, suggests that controlling surface run-off and spray drift (Schulz, 2004) can be particularly efficient for maintaining the desired state of macrophyte dominance. Potential measures restricting pollution of the water phase include buffer strips and sufficient riparian vegetation (Lorenz et al., 2022). Currently, it is suggested that buffer strips of approximately 5 m be used to protect lowland streams from agricultural run-off. However, a recent study by Vormeier et al. (2023) proposes that a width of up to 23 m may be necessary to safeguard aquatic macrophytes, due to the high mobility of herbicides in run-off. A generally stronger regulation of pesticide and fertilizer applications is needed because nitrate and pesticides transported via subsurface flow into sediments also impaired macrophyte growth in our study. Additionally, long-term storage of pesticides from former application can lead to highly polluted sediments, which then act as a significant source for pesticides. This exposure pathway must be considered additionally when restoring shallow aquatic systems to a macrophyte dominated state. Sediment removal may serve as a fitting restoration measure to address this aspect effectively. In order to prioritize management actions and ensure successful restoration resp. prevention of deterioration, it is crucial to gain comprehensive understanding of the indirect effects and interaction patterns among multiple stressors. Addressing these knowledge gaps will contribute to more effective decision-making and ultimately support the successful restoration or macrophyte-dominated ecosystems.

AUTHOR CONTRIBUTIONS

Bastian H. Polst: Conceptualization, methodology, investigation, formal analysis, writing—original draft & review/editing, visualization. **Joey Allen**: Conceptualization, investigation, formal analysis, writing—original draft & review/editing. **Franz Hölker**: Conceptualization, writing—review & editing, funding acquisition.

Sabine Hilt: Conceptualization, writing—review & editing, funding acquisition. **Herwig Stibor**: Conceptualization, writing—review & editing, funding acquisition. **Elisabeth M. Gross**: Conceptualization, writing—review & editing, funding acquisition, project coordination. **Mechthild Schmitt-Jansen**: Conceptualization, writing—original draft & review/editing, supervision, funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available at the Open Science Framework https://doi.org/10.17605/OSF.IO/8ZGS5 (Polst et al., 2023).

ORCID

Bastian H. Polst https://orcid.org/0000-0001-6499-8816
Joey Allen https://orcid.org/0000-0003-3075-8560
Franz Hölker https://orcid.org/0000-0001-5932-266X
Sabine Hilt https://orcid.org/0000-0002-0585-2822
Herwig Stibor https://orcid.org/0000-0001-5918-6020
Elisabeth M. Gross https://orcid.org/0000-0002-9392-8582
Mechthild Schmitt-Jansen https://orcid.org/0000-0002-4541-6515

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix SF1: Light availability (daily average per microcosm; two microcosms per treatment) at the bottom of the microcosm throughout the experiment measured with Hobo light data loggers. Treatments: green = control (C), orange = S 1 (sediment exposed ARO 1), blue = W 1 (water exposed ARO 1), pink = S 4 (sediment exposed ARO 4), yellow = W 4 (water exposed ARO 4).

Appendix SF2: N-NO_3 concentrations in the water column for ambient (blue dots) and heated (red dots) conditions at different time points through the experiment (T0 = day of treatment application, T1 = within the first week, Tx = xth week, T5 = during final sampling). Treatments: S 1 = sediment exposed ARO 1, W 1 = water exposed ARO 1, S 4 = sediment exposed ARO 4, W 4 = water exposed ARO4.

Appendix SF3: Organic pesticide concentrations at the end of the experiment measured in the water column (blue) and sediment porewater (brown). Treatments: S 1 = sediment exposed ARO 1, W 1 = water exposed ARO 1, S 4 = sediment exposed ARO 4, W 4 = water exposed ARO4.

Appendix SF4: Pesticide concentrations (red = terbuthylazine, green = pirimicarb, blue = tebuconazole) in the apical 10 cm of *Myriophyllum spicatum* at the end of the experiment. Plant biomass from the control treatments were used to test the recovery of pesticides of the extraction method and are therefore not available but control microcosms were previously found to be unimpacted by pesticides. Letter coding based on Dunn's post-hoc test ($p < 0.05$) and applies to the three pesticides separately as indicated by the respective colours. Treatments: S 1 = sediment exposed ARO 1, W 1 = water exposed ARO 1, S 4 = sediment exposed ARO 4, W 4 = water exposed ARO4.

Appendix SF5: Effect sizes (Hedges' *g*) for effects on the three macrophyte species *P. perfoliatus*, *M. spicatum* and *E. nuttallii*.

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