Warming lowers critical thresholds for multiple stressor–induced shifts between aquatic primary producers

Bastian H. Polst[1], Sabine Hilt[2], Herwig Stibor[3], Franz Hölker[2], Joey Allen[4,5], Vinita Vijayaraj[4], Nora Kipferler[3], Joséphine Leflaive[5], Elisabeth M. Gross[4], Mechthild Schmitt-Jansen[1]

[1] Helmholtz-Centre for Environmental Research – UFZ, Leipzig, Germany
[2] Leibnitz Institute for Freshwater Ecology and Inland Fisheries (IGB), Berlin, Germany
[3] Ludwig-Maximilians University Munich, Aquatic Ecology, Munich, Germany
[4] Université de Lorraine, LIEC UMR 7360 CNRS, Metz, France
[5] Université de Toulouse, ECOLAB UMR 5245 CNRS, Toulouse, France
E-mail contact: bastian-herbert.polst@ufz.de ; mechthild.schmitt@ufz.de

---

Keywords: Agricultural run-off, global warming, phototrophic aquatic communities, regime shift, safe operating space, multiple stressors

Abstract

In aquatic ecosystems, excessive nutrient loading is a global problem that can induce regime shifts from macrophyte- to phytoplankton-dominated states with severe consequences for ecosystem functions. Most agricultural landscapes are sites of nutrient and pesticide loading, which can interact with other stressors (e.g., warming) in additive, antagonistic, synergistic or reversed forms. The effects of multiple stressors on the resilience of macrophyte-dominated states and on critical thresholds for regime shifts are, however, unknown. We test the effects of individual and combined stressors of warming, nitrate, and various pesticides typically found in agricultural run-off (ARO) on the growth of macrophytes, periphyton, and phytoplankton in microcosms. We applied a one-level replicated design to test whether ARO induces a regime shift and a multifactorial dose–response design to model stressor thresholds and disentangle stressor interactions along a gradient. The individual stressors did not induce a regime shift, but the full ARO did. Nitrate and pesticides acted synergistically, inducing a shift with increasing phytoplankton biomass and decreasing macrophyte biomass. Warming amplified this effect and lowered critical thresholds for regime shifts. Shallow aquatic ecosystems in agricultural landscapes affected by global warming thus increasingly risk shifting to a turbid, phytoplankton-dominated state, and negatively impacting ecosystem service provisioning. Multiple stressor interactions must be considered when defining safe operating spaces for aquatic systems.

1. Introduction

In recent decades, the quantity and magnitude of co-occurring anthropogenic stressors on aquatic ecosystems have increased, particularly in agricultural landscapes (e.g., Schinegger et al., 2012). High nutrient loading from agriculture is the most widely occurring anthropogenic stressor (Rücker et al., 2019) and often co-occurs with a variety of pesticides (Halbach et al., 2021; Wijewardene et al., 2021). A recent meta-analysis reports that more than two-thirds of aquatic freshwater systems suffer from high nutrient loading, and co-occur with toxic pollution in 10-15% of cases (Nõges et al., 2016). These stressors can interact in complex ways, resulting in additive (the sum of individual stressor effects), antagonistic (combined effect lower than sum of individual stressor effects), synergistic (combined effect higher than the sum of individual stressor effects) or even reversed (change in effect direction) effects (Côté et al., 2016; Jackson et al., 2016). However, interaction patterns may vary along stressor types, stressor gradients and ecosystem complexity (Côté et al., 2016). Non-linear responses along gradients of stressors are the rule rather than the exception in ecosystems (Wagenhoff et al., 2011). The non-linear nature of several ecosystem responses further complicates the definition of related ecological thresholds. Threshold values are needed to compare interacting stressor effects and to define safe-operating-spaces for improved management (Pirotta et al., 2022; Scheffer et al., 2015).

Prominent examples for non-linear ecosystem dynamics are regime shifts from macrophyte-dominated to phytoplankton-dominated states in shallow aquatic lakes and ponds along gradients of nutrient loading (Scheffer et al., 1993). Shallow aquatic ecosystems are abundant across systems and biomes (Cael et al., 2017; Verpoorter et al., 2014), and provide important ecosystem functions and services (Hilt et al., 2017; Janssen et al., 2021). When critical nutrient threshold levels are exceeded, phytoplankton or periphyton shade out macrophytes (Olsen et al., 2015; Phillips et al., 2016). Combined with pesticides, nutrients loadings can still lead to phytoplankton blooms (Allen et al. 2021) and thus potentially induce regime shifts. Yet it remains unclear how the combined stressors in agricultural run-off interact and if the presence of pesticides modifies the threshold of nutrient induced regime shifts.

In addition to local stressors including agricultural run-off, elevated water temperatures caused by global warming, both long-term gradual increase as well as heatwaves, challenge our ecosystems more frequently, in future (Woolway et al., 2021). While higher temperatures generally increase the overall metabolism of organisms and lead to elevated growth or abundance, species differ in their optimal temperature ranges (Hansson et al., 2020; Odum et al., 1979). In shallow aquatic systems, phytoplankton dominance, and particularly cyanobacteria blooms, are projected to increase with rising temperatures (Jöhnk et al., 2008; Mooij et al., 2007; Paerl and Huisman, 2008). In general, global warming and eutrophication in freshwaters may mutually reinforce their effects (Moss et al., 2011). Furthermore, in combination with toxic stressors, warming can dampen the effect of these toxic stressors on algae (Chalifour and Juneau, 2011; Larras et al., 2013) and may shift the critical effect thresholds for herbicides. When combined with nutrient loading as presumably antagonistic stressor, elevated temperature may decrease the effect of one of the two stressors, and may lower the threshold for the stressor mixture.

Thresholds in non-linear systems such as regime shifts can be quantified by testing the response along a gradient of stressors, as recommended by Kreyling et al. (2018) even at the cost of further replication. Replicated approaches with fewer concentration levels neglect non-linear responses and hardly enable modelling of critical thresholds. In this study, we combined both approaches (replicated vs gradient design) to investigate whether warming modifies the critical thresholds for regime shifts between the dominance of different primary producers (macrophytes, phytoplankton, and periphyton) induced by multiple agricultural stressors. We built on previous experiments of Allen et al. (2021), who were testing the effects of agricultural run-off and warming on complex food web interactions including primary producers and consumers. Here we conducted two microcosm (8L) experiments simulating the primary producer level of typical fishless shallow freshwater ecosystems in agricultural landscapes. In a replicated approach, we compared controls with one level of mixed compounds representing agricultural run-off. In a multi-factorial gradient design we determined thresholds for regime shifts and tested the effect of warming on these thresholds. Additionally, stressor interactions were classified.

We hypothesized that 1) combined stressors (nitrate and a representative pesticide mixture) induce shifts from macrophyte- to phytoplankton- dominance, 2) elevated temperature lowers critical thresholds for multiple stressor-induced regime shifts, 3) co-occuring stressors (pesticides, nitrate, and elevated temperature) amplify the mechanisms causing regime shifts and result in synergistic stressor interactions.

2. Material and Methods

Two experiments were performed (Figure 1). The first experiment focused on the first hypothesis: combined stressors representative for agricultural run-off (ARO) induce regime shifts. The second experiment was performed to disentangle the relevance of individual stressors, to identify stressor patterns and to enable modelling of thresholds. This experiment had a more complex design and partly used a gradient approach on costs of replicates. Both experiments were performed with a comparable microcosm setup, based on Allen et al. (2021).

2.1 Microcosms

Microcosms (8 L, cylindrical glass vases, diameter: 25 cm, height: 40 cm) were set up with three macrophyte species typical for shallow aquatic ecosystems, *Potamogeton perfoliatus*, *Myriophyllum spicatum,* and *Elodea nuttallii*, as well as planktonic and benthic microalgal species. Algae species used in the replicated experiment were sampled from local ponds and streams. Cultured algae were used for the gradient experiment to further reduce impacts of external factors and increase reproducibility. *Potamogeton perfoliatus* was collected from the Spree River near Mönchwinkel (Brandenburg, Germany). *Myriophyllum spicatum* was collected from a pond at the campus of Ludwig Maximilian University of Munich (LMU) in Martinsried–Planegg (Bavaria, Germany), and *Elodea nuttallii* was collected from a private pond (Bavaria, Germany) for the gradient and in Goitzsche Lake (Sachsen-Anhalt, Germany) for the replicated experiment.

In the replicated experiment, planktonic algae communities from local ponds were used amounting to a volume of $1x10^6$ μm^3 mL^{-1} per microcosm. Benthic communities were sampled from a nearby stream (2 cm^2 of stones per microcosm). In the gradient experiment cultured algae were used: Four preferably planktonic algae species (*Chroococcus minutus*, *Anabaena* PCC7120, *Desmodesmus subspicatus*, *Scenedesmus obliquus*) and five preferably benthic algae species (*Komvophoron sp*,

Uronema confervicolum, *Oedogonium sp.*, *Nitzschia palea*, *Gomphonema parvulum*) were grown individually in enriched (0.5x stock solution of WC medium) Volvic© mineral water (Danone Waters Deutschland GmbH, Germany) and were mixed in equal shares, amounting to $1x10^6$ μm^3 mL^{-1} each for planktonic and benthic algal cells as inoculum for the microcosms.

The sediment was prepared based on the OECD guideline 239 Water–Sediment *Myriophyllum spicatum* Toxicity Test (OECD, 2014). In short, we mixed 73.5% quartz sand (0.1–0.3 mm, Schicker Mineral, Germany), 20% Kaolin (Imerys, France), 5% peat (<1 mm, Klasmann–Deilman GmbH, Germany), 1% nettle powder obtained from a local field site presumably not affected by pesticides, and 0.5% $CaCO_3$ (Sigma-Aldric). Approximately 380 g sediment was prepared for each microcosm and placed in a glass bowl insert. The sediment was overlaid with a 2 cm quartz sand layer, watered with Volvic© water and placed in the dark for three days to give the sediment time to settle.

Apical macrophyte stems were cut at 10 cm lengths, and two stems per species were planted in the prepared sediment for each microcosm. Frosted polypropylene plastic strips (GBC, England) from the sediment up to the water surface provided a surface for periphyton development. The microcosms were filled with 8 L of Volvic© mineral water, and glass pipettes were inserted as outflow for aeration.

The microcosms were placed under LED light (mean 70 ± 12 µmol m^{-2} s^{-1}, Model C65 100 mA 5730, Valoya Oy, Finland) in a temperature-controlled laboratory at 16:8h light:dark cycle and the lower half of each microcosm was wrapped in dark foil to limit horizontal light input. The room temperature was set to 22 ± 0.5 °C. For the second experiment, microcosms undergoing temperature treatment were placed on 80-W heating mats (AccuLux, Germany) and controlled via a temperature-responsive dc outlet set to 26 ± 0.2°C (Shenzhen Inkbird Technology, China), which prevented overheating of the microcosms

2.2. Treatment setup in the replicated experiment

In the replicated experiment a mixture of terbuthylazine, pirimicarb, tebuconazole, $CuSO_4$, and KNO_3 (all manufactured by Sigma-Aldrich, USA), representing agricultural run-off (ARO) was added (similar to the approach used in Allen et al. (2021), see Figure 1): The pesticides, including copper sulphate, were selected as representatives of their respective pesticide group and are commonly

found in agriculturally impacted aquatic ecosystems (Halbach et al., 2021; Lefrancq et al., 2017; Wijewardene et al., 2021): herbicide (terbuthylazine), insecticide (pirimicarb), and fungicide (tebuconazole). Nitrate was selected for the nutrient treatment due to the high relevance in aquatic ecosystems nearby agricultural sites (e.g. James et al., 2005; Xu et al., 2014). The three organic pesticides were dissolved in dimethylsulfoxid (Sigma-Aldrich, USA; final concentration <0.01%); the other two components were dissolved in MilliQ water. One dose of this ARO mixture (3 µg L^{-1} Terbuthylazine, 15 µg L^{-1} Pirimicarb, 90 µg L^{-1} Tebuconazole, 42 µg L^{-1} Copper, 9000 µg L^{-1} N as Nitrate; SI Table1: Concentration C8) was compared to the control, both at a temperature of 22 °C. Replicates (n = 4) were used to account for variability.

2.3 Treatment setup of the gradient experiment

In the gradient experiment a multi-factorial dose–response design was used: treatments were tested individually and in combination (see Figure 1 & SI Table 1). Additionally, a dose–response design with a gradient of the respective chemical treatments was applied after validating consistent responses in the microcosms of the first experiment. The control was replicated (n = 5) to enable comparison with the first experiment while the actual treatments were stretched over a gradient in an enrichment factor of 2 (n = 1 per concentration). Six different ARO components or their mixture were tested at five different concentrations. Concentrations ranged from relative enrichment factor 1 (C1) to concentrations at a relative enrichment factor 16 (C16; SI Table 1) following a geometric progression in their relative enrichment factor (REF). Five control microcosms each, at ambient and elevated temperatures, were randomly distributed between treated microcosms. The chemicals were prepared and applied the same way as in the first experiment. The increase of +4°C in the heated microcosm refers to predicted climate-change-related temperature increases during heat waves. (Woolway et al., 2021).

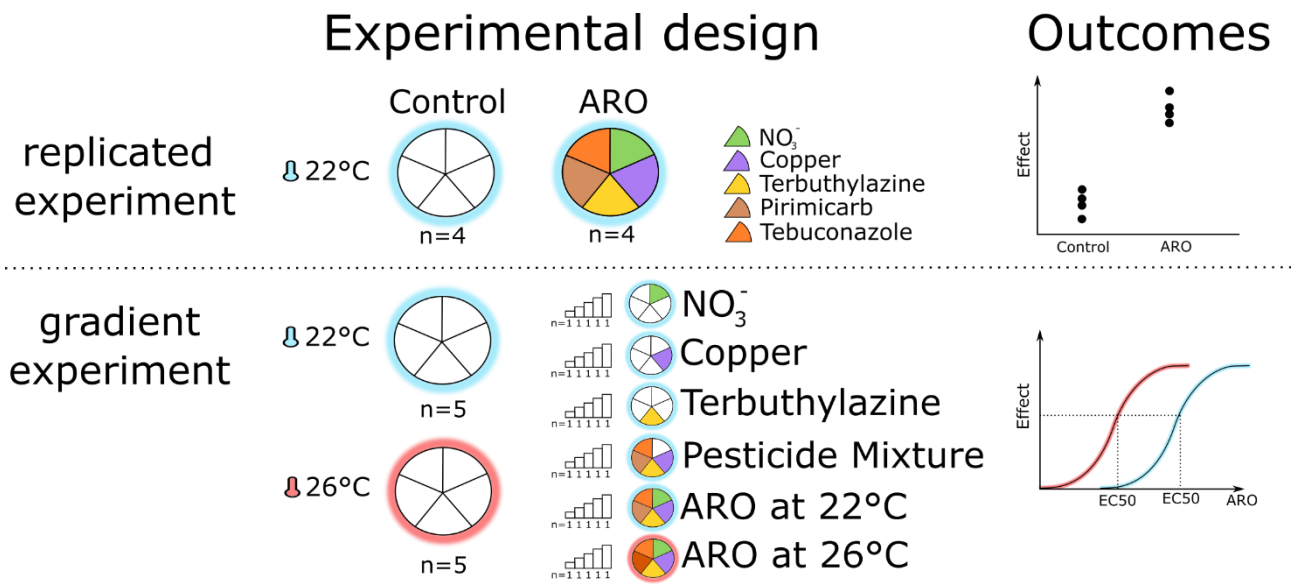


Figure 1: Design of our study: The replicated experiment tested a control (n=4) and one treatment level of agricultural run-off (ARO) (n = 4) containing nutrients and a mixture of pesticides (copper, terbuthylazine, pirimicarb, tebuconazole). The gradient experiment used two treatments at 22°C (control, n = 5) and 26°C (temperature, n = 5) without chemical contamination. Nitrate (NO_3), copper and terbuthylazine as well as a pesticide mixture were tested individually and in combination (ARO) along a gradient of five concentrations (n = 1). For details see SI Table 1.

2.4 Timeline of the experiments

The microcosms were filled with 8 L of Volvic© water, directly inoculated with the planktonic and benthic algae, and given two days to acclimate to experimental conditions. The glass inserts with sediment and macrophytes were placed in the microcosms and given three days to acclimate before the treatments (addition of chemical stressors and warming) were applied. A low dose of macronutrients (224 µg L^{-1} N as KNO_3, 31 µg L^{-1} as KH_2PO_4) was added thrice a week, and evaporated water was replaced with distilled water. Temperature was measured daily, pH-value was measured once a week. In the replicated experiment light availability at the bottom of the experiment was monitored during the experiment using data loggers (HoBo light logger, Onset Computer Corporation, USA). Samples for pigment analysis of phytoplankton communities were taken weekly (replicated experiment) resp. biweekly (gradient experiment). The replicated and the gradient experiments lasted for four and six weeks after the start of treatment exposure, respectively. At the end of both experiments macrophytes and periphyton were sampled.

2.5 Biomass of primary producers

Phytoplankton samples were filtered (0.7 µm glass-fibre filters, Labsolute, Germany) for dry weight and pigment analysis (see SI). For phytoplankton chlorophyll a was preferred as a surrogate of biomass for phytoplankton due to the possibility of resuspended detritus from the microcosm bottom when handling the microcosm for sampling. At the end of both experiments, individual macrophyte species were collected separately. Macrophytes were dried at 55°C for 48 h and weighed thus obtaining their biomass. Periphyton was brushed off the plastic strips (135 cm^2) using toothbrushes and resuspended in 100 mL Volvic© mineral water. Periphyton suspensions were then filtered (0.7 µm Microfiber, Labsolute, Germany) for dry weight (55°C for 24 h) and pigment analysis (see SI). For further analysis periphyton dry weight was chosen to attribute for the biofilm matrix and its contribution to possible shading effects on macrophytes.

2.6 Pesticide and nutrient analyses

In the replicated experiment, water samples were taken to determine the real concentrations of pesticides at the start and the end of the experiment after four weeks. In the gradient experiment, water samples were taken one hour after addition, and then two, four and six weeks later. Samples were filtered (0.2 µm cellulose acetate filter, Labsolute, Germany) and either frozen until further analysis at -20°C (pesticides) or measured directly (nutrients). Pesticides were measured with an LTQ-OrbiTrap (see SI; Thermo Scientific, USA). Dissolved inorganic nutrients (PO_4^{3-}, NO_3, NH_3, NH_4^-) sampled at the start and the end of the experiments were measured according to DIN-EN-26777 (1993), DIN-EN-ISO 13395 (1996) and DIN-EN-ISO-6878 (2004). Copper samples were measured according to Vijayaraj et al. (2022a).

2.7 Statistical evaluation

For statistical analysis of the replicated treatments, t-tests were used for comparing biomass data. Effect sizes were used in both experiments for comparison of the strength and directionality of the response to the different stressors. The total dry weight (all macrophyte species accumulated; periphyton) and phytoplankton chlorophyll a (chl *a*) at its peak concentration during the experiment was used to calculate effect sizes in the replicated experiment. The same analysis was performed for the second experiment. Additionally effect sizes based on dry weight for the individual macrophyte species and for periphyton were calculated. In both experiments the effect sizes were

calculated as Glass's delta (Fritz et al., 2012; Glass, 1976). Due to our dose-response design in the second experiment, there was no standard deviation for the single treatments along the gradient (n = 1), but for the control treatments (n=5). Making use of the standard deviation from the control treatment enabled effect size statistics according to Glass (1976). Glass's delta substitutes the non-existing standard deviation of the non-control treatments by the standard deviation of the control treatment and leads to more robust results. This approach is backed by low variability in results obtained from the first experiment (coefficient of variation of effect sizes in the ARO treatment ~0.15 for macrophytes and phytoplankton, see Figure 2). The mean of the control treatment ($M_{control}$), its standard deviation ($SD_{Control}$) and the single data value of the respective treatment (M_T) were considered in the equation:

$$\text{Glass's } \Delta = \frac{M_T - M_{control}}{SD_{control}}$$

An effect size Glass's Δ of 1 indicates a positive effect equivalent to the size of the standard deviation of the control treatment, and *visa-versa* for a negative effect (Δ = -1). Effect sizes between 1 and -1 were within the standard deviation of the control treatment data, and therefore these data points show no effect by definition. Values higher than 1 indicate an effect that is more than one standard deviation greater than the control treatment*. Vice versa,* a value lower than -1 indicates an effect that is more than one standard deviation lower than the control treatment. For this study, effect sizes equal or higher 1 were considered as positive effect, effect sizes equal or lower than -1 were considered as negative effect. This is considered to be a conservative approach compared to common effect-size assessments using lower limits (e.g. 0.5 for *medium* effects; Sawilowsky, 2009). This approach was further supported by one-sample t-tests comparing the individual biomass data of each treatment to the ones of the replicated controls. We considered a "shift" from macrophyte- to phytoplankton-dominance as having occurred when the effect size of accumulated macrophyte dry weight was less than or equal to -1 and the effect size for phytoplankton biomass was greater than or equal to 1 at the same time, meaning that both compartments showed a clear but contrasting effect in their biomass data (final dry weight for macrophytes and periphyton, peak chl *a* for phytoplankton to account for delayed effects).

In the gradient study, the statistical power derives from the distribution of samples along concentrations in combination with a modelling approach: Effective concentrations of selected percentiles, e.g. the effective concentration for 50% quantile (EC50), and their error margin, e.g. the standard error, can be modelled and used to compare thresholds in a statistical valid way. Threshold values allow for quantification and further comparison of the observed effects along the gradient. To derive these threshold values, dose–response curves were fitted based on the four-parametric log-logistic models using the *drc* package (v3.0-1, Ritz et al. (2015)) for R (R Core Team, 2020) for the biomass data (dry weight for macrophytes and periphyton; peak chlorophyll a for phytoplankton). To allow for relative comparison of these values we fixed the upper and lower limits of the four-parametric models to the observed carrying capacity of our microcosms: the mean of the control treatment as well as the highest (phytoplankton) and lowest (macrophytes) biomass values observed in our experiment across all treatments (see Table SI 6). For comparison of thresholds between treatments, we choose the EC50-values as a robust descriptor of the response. The modelled EC50-values were tested for significant differences using the *drc* package (Ritz et al., 2015).

While the biomass of macrophytes and periphyton at the end of the experiment were used for correlation analysis, the peak phytoplankton biomass represented as chlorophyll a from the three time points during the experiment was used. Correlation tests (Pearson's r) were performed using the statistical software R (R Core Team, 2020) to indicate possible interactions between the primary producers, e.g. shading.

To identify and compare stressor interactions for different treatments, concentrations, and phototrophic compartments (macrophytes, phytoplankton, periphyton), stressor interaction types were classified by comparing additive stressor effects (calculated effects based on individual stressor effects) and the observed combined stressor effects. Effect size data from the second experiment were used to compare the calculated stressor addition with the observed stressor effects: to account for uncertainty due to methodological errors and background noise, we use a conservative approach considering a ± 10% margin of the higher absolute value of both stressors. If the difference between the calculated and observed stressor effects was within this range, we classified the interaction pattern as an additive effect. Outside of this range, three types of non-additive stressor interactions

were assigned according to Côté et al. (2016) and Jackson et al. (2016): antagonistic (combined effect lower than sum of individual stressor effects), synergistic (combined effect higher than sum of individual stressor effects), or reversed interactions (change in effect direction).

3. Results

3.1. Physico-chemical parameters

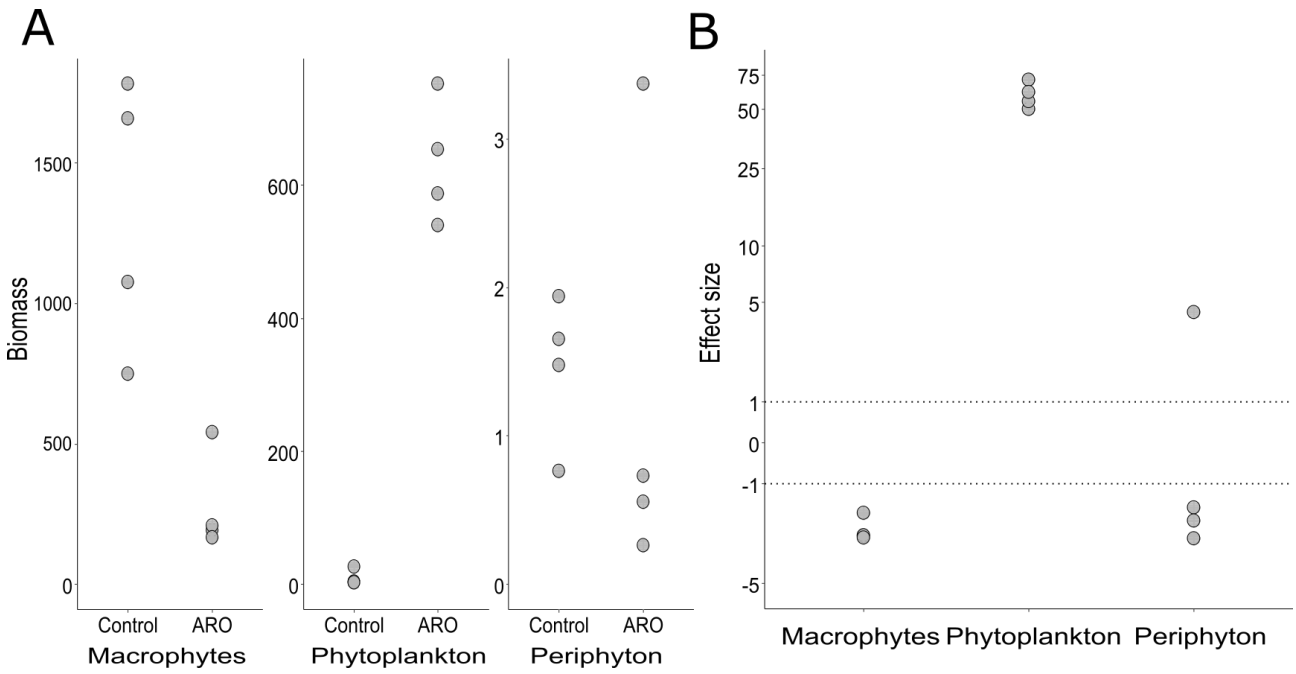
Nitrate was within the nominal concentrations of the treatments at the beginning of both experiments (1 h after start) and depleted along with the concentrations of other nutrients during the experiments (see SI Figure 1). In the replicate experiment, organic pesticide and nutrient concentrations were slightly above (~110%) the nominal concentrations at the start of the experiment while they were slightly below (~80%) nominal concentrations in the gradient experiment and decreased throughout both experiments. At the end of the replicate experiment (after four weeks), approximately 50% of Terbuthylazine, 25% of Pirimicarb and 60% of Tebuconazole were still present, whereas only approximately 10% of the pesticides were present at the end of the gradient experiment after six weeks (see SI Figure 2). Copper values reached approximately 50% of the nominal concentrations at the start of the experiment. The pH-value ranged between 8 and 9 with small treatment-related differences in the gradient experiment only. Throughout the experiments, water temperature stayed within ± 0.5°C of the desired value for both temperature treatments. Light measured at the bottom of the microcosm in the replicated experiment shows higher light availability in the control (e.g. 22 µmol s^{-1} m^{-2} resp. ~30% of surface light at day 15) compared to the ARO treatment (e.g. 8 µmol s^{-1} m^{-2} resp. 11% of surface light at day 15).

3.2. Effects of the agricultural run-off mixture in the replicate experiment

Macrophyte biomass (dry weight) was significantly lower in the ARO treatment (227 ± 176 mg) compared to the control (1315 ± 487 mg) ($p < 0.001$, Figure 2). Their effect size averaged at -2.5 ± 0.4. Phytoplankton showed a significant increase in the ARO treatment (633 ± 80 µg L^{-1} chl *a*) compared to the controls (9 ± 10.5 µg L^{-1} chl *a*) with an effect size up to 75 ($p < 0.001$, Figure 2). No significant unidirectional response of periphyton was observed. Periphyton effect size values ranged from -2.8 to 4.4 (Figure 2), showing clear effects (effect size >1 resp. Δ<-1) in the individual

298 microcosms but not when averaged across the replicates (-0.5 ± 3.3). A clear shift from macrophyte
299 to phytoplankton dominance was observed in all microcosms.

300



301

302 Figure 2: A) Biomasses for macrophytes (dry weight in mg), phytoplankton (chl a in µg L^{-1}) and periphyton (dry weight in g
303 m^{-2}) and B) Effect sizes (Glass's delta) for macrophytes (dry weight), phytoplankton (chl a) and periphyton (dry weight)
304 after exposure to agricultural run-off for 4 weeks in the replicated experiment (n = 4).

305

306 *3.3 Gradient experiment*

307 *3.3.1 Effects of individual and combined as agricultural run-off (ARO)*

308 The final macrophyte aboveground biomass showed no clear trend for the individual stressors or for
309 the combined pesticide treatment (Figure 3). However, individual species responded differentially,
310 especially *P. perfoliatus* showed trends for various stressor treatments (see SI Figure 4). Only the
311 nitrate treatment resulted partly in negative effects on the accumulated macrophyte biomass (Figure
312 4 & SI Figure 4): While the highest nitrate concentration leads to a significant effect according to the
313 t-test but not with regard to the effect sizes, the assessment of a shift for this concentration remains
314 unclear. However, no meaningful EC50 for the final aboveground macrophyte biomass could be
315 modelled using a log-logistic model for any of the individual stressors or the combined pesticides
316 due to the lack of effects.

Phytoplankton biomass, in contrast, showed a positive response to all nitrate concentrations after 11 days, with a strong increase at the third concentration and above (Figure 3), but no response to other treatments. Therefore, a full dose–response curve could be modelled for phytoplankton and revealed an EC50-value of 11.3 ± 5.8 REF (standard error (SE); Figure 4). Periphyton biomass showed changes but no clear trends due to the high variability in the control samples (see SI Figure 4). According to our definition of shifts (positive effect in phytoplankton and negative effect in macrophytes), only the intermediate nitrate exposure concentration (C4) led to a shift from macrophyte dominance to phytoplankton dominance in the single-stressor and the combined pesticide treatments (Figure 3).

In the treatment combining all pesticides with nitrate exposed at ambient temperature (22 °C), negative effects were observed at the two highest exposure concentrations for the accumulated macrophyte biomass (Figure 3). The EC50-value derived from the modelling approach of the accumulated macrophyte biomass in the ARO treatment (7.3 ± 2.7 REF, SE, Figure 4) indicates a stronger effect than for the nitrate treatment. In contrast to the negative effects on macrophytes, a positive effect was observed for phytoplankton during the first half of the experiment, even at a low dose. The phytoplankton the EC50-value for the ARO treatment (11.0 ± 4.1 REF, SE, Figure 4) was not significantly different from that of the nitrate treatment. A shift from macrophyte to phytoplankton dominance was found for the two highest ARO exposure concentrations.

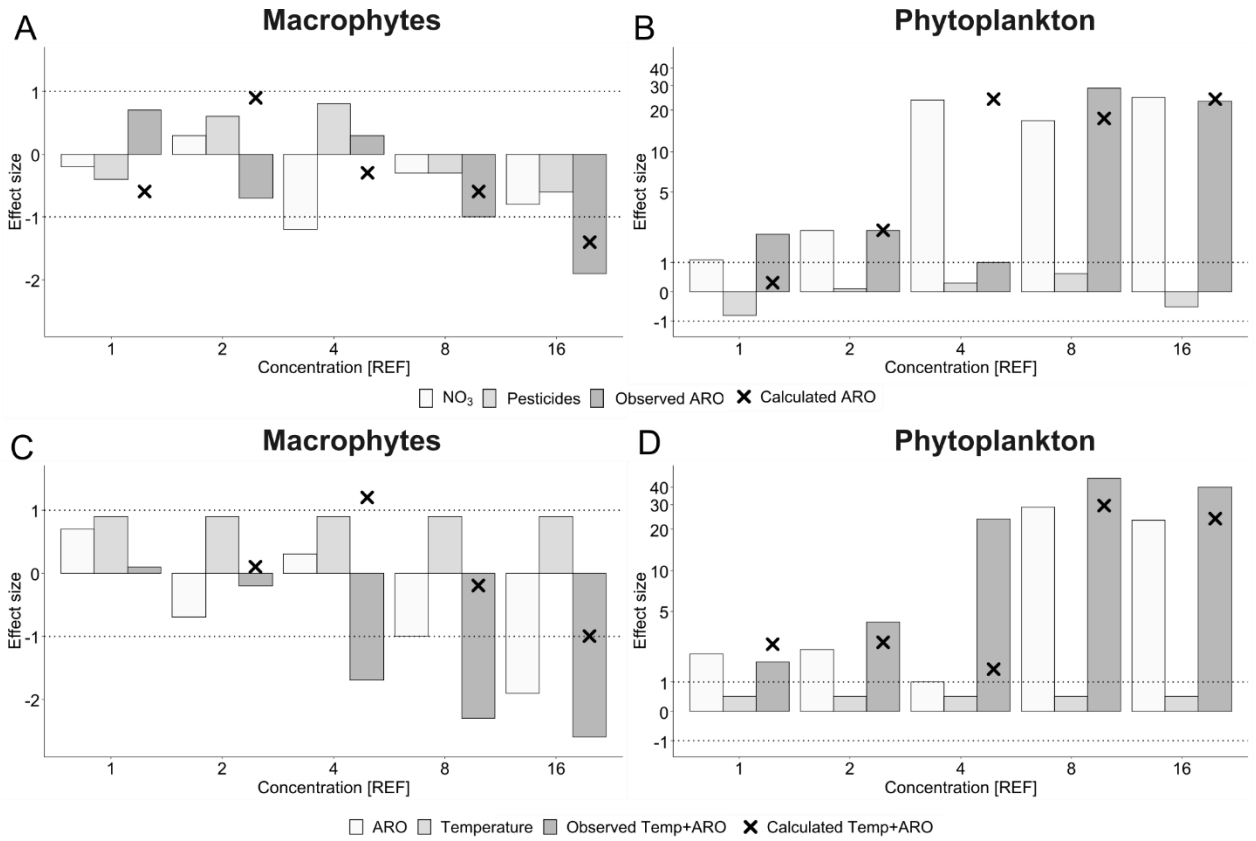


Figure 3: Effect sizes (Glass's delta) at the end of the experiment for macrophytes (A & C) and of the phytoplankton biomass during its peak in the second week of the experiment (B & D). The response to the nitrate (NO_3), the combined pesticide treatment (Pesticides), their calculated additive effect (black cross) and their observed interactive effect (observed ARO) along a gradient of 5 concentrations for each treatment (A & B). The response to the ARO and the temperature treatment, their calculated additive effect (black cross) and their observed interactive effect (observed Temp+ARO). Exposure concentrations are given as relative enrichment factor (REF, see SI Table 1 for stressor concentrations).

3.3.2 Effects of increased temperature, individually and combined with ARO

On average, increased temperature (26 °C) alone had no positive effect on the accumulated macrophyte biomass (Figure 3). Diverse effects were observed for individual macrophyte species, e.g. only *P. perfoliatus* responded positively to warming (see SI Figure 4). No effects were observed for periphyton and phytoplankton. Elevated temperature alone did not induce a shift from macrophyte to phytoplankton dominance.

The combination of all stressors including elevated temperature affected macrophyte biomass negatively and amplified the effects already observed for the ARO treatments at low temperature (Figure 3). The EC50-value for the accumulated macrophyte biomass shifted towards a lower concentration (2.7 ± 0.3 REF, SE, Figure 4) in comparison to effect values for the ARO treatment without warming (7.3 ± 2.7 REF, SE, Figure 4). The same shift was observed for phytoplankton

EC50-values (Temp+ARO: 3.9 ± 0.3 REF; ARO: 11.0 ± 4.1 REF, SE, Figure 4). Early phytoplankton development showed a stronger response in the heated treatments than in any other treatment at each concentration level. Periphyton showed no consistent response patterns (see SI Figure 4). Ultimately, a shift was found for the third to the highest ARO exposure concentrations.

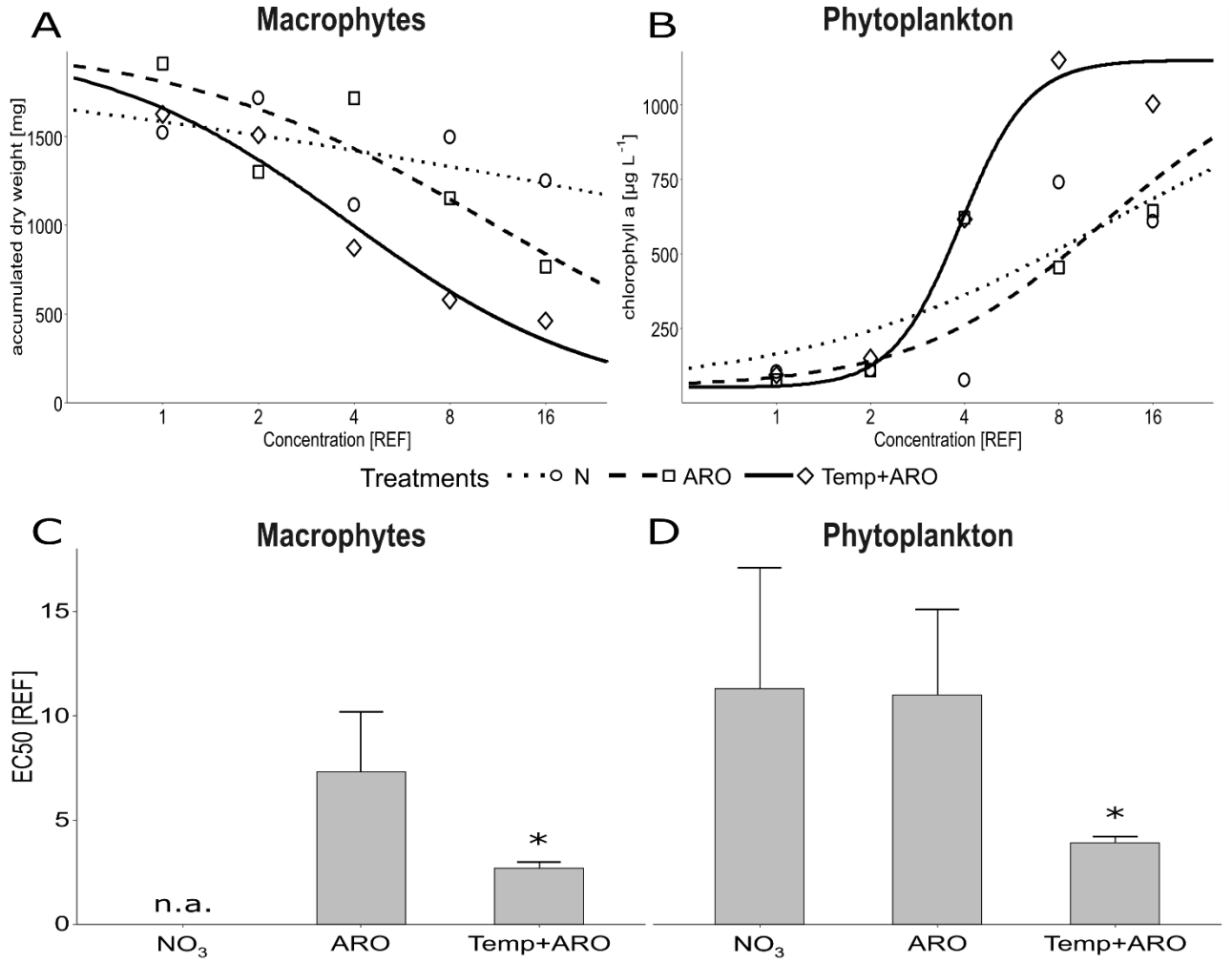


Figure 4: Dose Response curves modelled based on the biomass data for A) macrophytes (final dry weight) and B) phytoplankton (peak chl *a*) as well as their respective EC50 values C) for macrophytes and D) for phytoplankton. EC50-values (± standard error) derived from log-logistic modelling of the gradient studies and their standard errors. Data are given for the accumulated macrophyte and phytoplankton biomass in the treatments containing only nitrate (NO_3), the full mixture of agricultural run-off (ARO) containing nitrate, several pesticides and copper, and ARO in combination with warming from 22°C to 26 °C (Temp+ARO). EC50-values could not be modelled for the macrophyte biomass in the nitrate treatment due to effects lower than 50 %. Asterisks (*) indicate a significant difference ($p < 0.05$) at the treatment combining warming with the ARO to the other treatments for both, macrophytes and phytoplankton biomass, respectively. For the nitrate (NO_3) treatment, no meaningful EC50 could be modelled (n.a.).

3.3.3 Stressor interaction patterns

3.3.3.1 Interactions between pesticide mixture and nitrate

Synergistic interactions affecting the macrophyte biomass were found at higher exposure concentrations, as biomass declined more strongly than would be expected from addition of the individual stressor effects (Figure 3). At lower and intermediate concentrations, the effects were too weak to be classified as interaction types (within the set limits of -1 or 1). Stressor interactions affected the individual macrophyte species differently, with reversed interactions for *P. perfoliatus* and antagonistic interactions for *E. nuttallii* and *M. spicatum* (see SI Table 4). Phytoplankton showed various responses to stressor interactions, with one synergistic interaction and one additive effect at the highest concentrations (Figure 3, see SI Table 4). There was a remarkable response at the third concentration (C4, Figure 3), as the observed interaction was considerably lower than the calculated value, resulting in a strong antagonistic effect. The stressors mostly showed antagonistic interactions for the periphyton (see SI Table 4).

3.3.3.2 Interactions between temperature and ARO

For the accumulated macrophyte biomass, synergistic interactions were found at higher ARO exposure concentrations (Figure 3, see SI Table 5). *Potamogeton perfoliatus* showed reversed interactions while *M. spicatum* showed both antagonistic and synergistic interactions, depending on the ARO concentration. For *E. nuttallii,* all possible interaction types were found. Antagonistic interactions were prevalent for periphyton, while synergistic interactions dominated for phytoplankton, with the strongest synergistic interactions at the third exposure concentration (Figure 3). Here, one stressor had an effect size below 1, which we did not consider to be significant, and the other stressor had only a rather weak effect with a value of about 1. Yet the combination of all stressors led to an effect size above 20 and a huge discrepancy compared with the calculated additive effect at the third concentration.

3.3.4 Correlation analysis of biomass of autotrophic compartments

Finally, accumulated macrophyte biomass correlated negatively and significantly ($p<0.05$) with phytoplankton biomass in the second and fourth week of the experiment (Pearson's r: -0.79 and -0.53, respectively; see SI Table 3). The correlation at the end of the gradient experiment (sixth week) was not significant. Periphyton showed no significant correlation with the other primary producers.

4. Discussion

Combined stressors from agricultural run-off (nitrate and representative pesticides) severely affect aquatic primary producers and their competition. As periphyton biomass was highly variable (probably due to the influence of phytoplankton shading, detritus and micrograzers), we focus on macrophyte–phytoplankton relationships. Combined stressors induced regime shifts between the dominance of primary producers in our experimental systems, which mimic simplified shallow aquatic ecosystems. Warming amplified the observed effects and lowered the critical thresholds for regime shifts in ARO treatments.

Scale-dependency may impact a direct transfer of these microcosm results to the field: Shading effects of phytoplankton on macrophytes can be stronger at higher water depth, and less nutrients are locked in periphyton growing on the microcosm walls (“wall effect”). On the other hand, effects are expected to be masked to a greater extent in more complex *in-situ* contexts (Vijayaraj et al., 2022b). Despite these differences to field situations the mechanisms revealed for stressor interactions in this proof-of-principle study could only be disentangled by factorial experimental designs and are expected to be comparable along scales. However, final proof of upscaling needs confirmation of derived hypothesis from experiments in the field.

4.1 Comparison of the results from the replicated and the gradient approach

To disentangle multiple stressor effects on regime shifts, we combined a replicated and a gradient experimental approach. While our replicated experiment proved significant biomass changes in the combined stressor treatment, the gradient approach showed a dose-dependency and revealed thresholds for the observed effects of single and combined stressors. Both experimental designs revealed comparable results showing a shift towards phytoplankton dominance despite slightly different experimental conditions which may limit comparison of both experiments. However, ARO effects were stronger in the replicated experiment due to differing temporal dynamics in the development of phytoplankton related to nutrient and pesticides concentrations. Some studies, e.g. Barker et al. (2008) and Rodrigo et al. (2017), use a replicated gradient design to model non-linear effects of macrophytes and to derive thresholds. Only Barker et al. (2008) have done this in a regime shift context.

The focus of our study was on the non-linear regime shifts, the response to warming and a potential change of interaction types between stressors. A gradient design was recommended by Kreyling et al. (2018) for these very reasons and enabled an estimate of thresholds additionally to the statistical proof of the phenomenon provided by the replicate experiment. For future studies we recommend at least five stressor levels resp. concentrations along the gradient to enable robust non-linear modelling by using the model applied in this study. However, the choice of model needs to be considered to define a minimum number of concentrations. Our hybrid study supported threshold modelling and shows that these kind of studies (including other stressors not tested in this study) are needed at larger scale (mesocosm & field studies) and complexity (trophic levels).

4.2 Combined agricultural stressors can induce regime shifts

The replicated experiment indicated a strong shift in dominance of primary producers when nitrate and pesticides were combined (ARO) at a high concentration supporting our first hypothesis. In our gradient experiment, this shift was already observed at half the ARO concentration tested in the replicated experiment, supporting findings by Allen et al. (2021) who found an increase in phytoplankton due to ARO exposure at similar ARO concentration. However, Allen et al. (2021) only found an increase in phytoplankton without an accompanying decline of macrophytes which may be explained by a longer acclimation time (17 days) for macrophytes before the treatment application. This time may have been sufficient for macrophytes to reach the water surface and avoid shading effects through phytoplankton. Initial conditions for macrophytes thus seem crucial for their response to multiple stressors. In our study the combination of nutrients and pesticides that have little to no effects when applied individually, initiated a decline of macrophytes, thus increases the risk for regime shifts between the dominance of different primary producers in shallow aquatic ecosystems. However, temporal differences like acclimation time and stressor depletion over time are crucial factors defining this risk.

4.3 No thresholds for individual stressors as they did not induce regime shifts

Contrary to our expectation, the addition of terbuthylazine or copper, individually, or of the pesticide mixture without nitrate did not negatively affect the growth of phytoplankton nor macrophytes or even increased biomass of individual species. This is in line with Coors et al. (2006), who found an

increase in dry weight of submerged macrophytes (including *M. spicatum*) at comparable concentrations of 5 µg L^{-1} terbuthylazine. Coutris et al. (2011) also showed that several macrophyte species (including *M. spicatum*) tolerate a herbicide mixture at concentrations of 6 µg L^{-1}, similar to those used in our study, and only decreased in biomass at concentrations as high as 60 µg L^{-1}. The lack of a response to copper in our study might be explained by a negative influence of pH or dissolved organic carbon on copper toxicity. Roussel et al. (2007) only found copper-induced effects on macrophytes in mesocosms at concentrations (75 µg L^{-1}) higher than applied in our experiment. In conclusion, our study cannot derive thresholds for safe operating spaces for regime shifts induced by pesticides.

Experiments with individual stressors revealed that only nitrate had a positive effect on phytoplankton growth. However, this effect was not sufficient to induce a regime shift along the whole gradient, as the macrophytes showed little or no response. Modelled thresholds for regime shifts (increase in phytoplankton biomass accompanied by a macrophyte decline) have been reported at 1.5 mg L^{-1} N-NO_3 (Barker et al., 2008). In tiered approaches these shifts were found at ≥2 mg L^{-1} total nitrogen (Sagrario et al., 2005) and ≥3.5 mg L^{-1} total nitrogen (Olsen et al., 2015). In our study phytoplankton increased already at the lowest concentration tested (1.1 mg L^{-1} N-NO_3) but showed a huge leap between 2.25 and 4.5 mg L^{-1} N-NO_3. However, no thresholds could be modelled for macrophyte biomass in the nitrate treatment. The small scale of our microcosms leading to fewer shading, an uptake of nutrients by wall periphyton, or phosphorus limitation as in the cited studies, may explain this difference.

Comparing the phytoplankton biomass in the nitrate and the ARO treatment, both show effects already at the lowest concentration. A leap towards higher phytoplankton biomass (effect size of ≥20) occurred at a lower concentration (4.5 mg L^{-1} N-NO_3) in comparison to the combined ARO treatment (9 mg L^{-1} N-NO_3). This difference is not reflected in the EC50-values, yet indicates the possibility of a modifying nitrate effect by the presence of pesticides.

Continuous warming of 4°C resulted in a species-specific temperature response. The effect (or lack thereof) on individual macrophyte species mostly aligns with other studies, confirming our findings (Allen et al., 2021; Hansson et al., 2020; Mckee et al., 2002; Zhang et al., 2019). Although no positive

485 effect of elevated temperature alone on phytoplankton biomass was observed in our and other
486 studies, Allen et al. (2021) and Hansson et al. (2020) found a change in phytoplankton diversity,
487 indicating a possible adaptation of the community to higher water temperature.

488 In summary, individual stressors did not show clear effects enabling reliable estimation of thresholds
489 for regime shifts along the concentration range chosen in this study. But for the combination of the
490 individual agricultural stressors a non-linear shift was observed, and a threshold value could be
491 modelled.

492 *4.4 Elevated temperature changes threshold concentrations of ARO for regime shifts*

493 Elevated water temperatures decreased thresholds for regime shifts by a factor of three to four,
494 confirming our second hypothesis. The accumulated macrophyte biomass decreased drastically at
495 elevated temperatures when combined with ARO. This could be attributed to the higher
496 phytoplankton biomass, which was observed for the ARO treatments at higher temperature. Allen et
497 al. (2021) did not observe a temperature-induced increase of phytoplankton biomass and no
498 decrease of macrophyte biomass when their systems were exposed to ARO at higher temperature,
499 probably due to nutrient limitation. However, other studies combining herbicides and elevated
500 temperature indicate reduced sensitivity of algae (Chalifour and Juneau, 2011; Larras et al., 2013;
501 Tasmin et al., 2014) or a stronger increase in phytoplankton biomass at higher temperatures
502 (Verbeek et al. 2018) and thus support our findings. For the interaction of nutrients and warming
503 mostly synergistic interactions for phytoplankton are reported but interaction types may differ
504 depending on trophic states, the carrying capacity and the species present in the ecosystem (Lürling
505 et al., 2013; Richardson et al., 2019; Rigosi et al., 2014). Thus both the antagonistic interaction of
506 pesticides and higher temperatures as well as synergistic interaction of nutrients and higher
507 temperatures support the lower thresholds for regime shifts. Our study thus suggests that global
508 warming further increases the risk of shifts from clear-water macrophyte dominance to turbid,
509 phytoplankton-dominated conditions in aquatic ecosystems exposed to agricultural run-off
510 containing nutrients and pesticides.

511

4.5 Mechanism leading to the observed shifts

Microalgae are more sensitive towards pesticides than macrophytes (Giddings et al., 2013), giving them a disadvantage when competing in a pesticide rich environment. In our study, initially strong phytoplankton development due to high nitrate concentrations and light limitation for macrophytes is assumed to be responsible for the observed regime shifts (Jackson, 2003; Le Bagousse-Pinguet et al., 2012). Light limitation is the main mechanism for macrophyte decline and regime shifts (Scheffer et al. 1993, Le Bagousse-Pinguet et al, 2012) and treatment-related differences in light availability were observed in the replicated experiment. Various mechanisms on different scales could have contributed further: On community level the phytoplankton may have adapted to herbicide pollution through selection of tolerant species (Blanck, 2002; Christensen et al., 2006); on cellular level higher temperatures further increase nutrient uptake efficiency and detoxification rates (Chalifour and Juneau, 2011; Jensen and Andersen, 1992; Olsen et al., 2017). Remarkably, the negative effect on macrophytes at the end of the gradient experiment was found despite the crash of phytoplankton halfway during our experiment, indicating a long-lasting or time-delayed effect from phytoplankton blooms two weeks before.

4.5 Synergistic stressor interactions characterize the regime shifts

Synergistic interactions dominated in both of our tested stressor combinations: pesticides and nitrate (ARO) and the same at elevated temperatures (ARO+Temp). This confirms our third hypothesis, but partially contradicts findings of previous meta-analyses. Côté et al. (2016) reported mainly antagonistic interactions at the ecosystem level in aquatic and terrestrial systems, and Jackson et al. (2016) found equal shares of antagonistic and synergistic interactions in 616 and 88 studies at the community and ecosystem level in freshwater systems, respectively. However, Crain et al. (2008) conclude from a meta-analysis of 171 studies focusing on marine ecosystems that an increasing number of stressors leads to more synergistic interactions confirming our findings. Côté et al. (2016) concluded that the interaction types are highly dependent on the biological observation parameter, the taxonomic group, and the biological organisation level which we can confirm when comparing the response of single macrophyte species with total macrophyte biomass.

Reversed interactions have rarely been reported in literature, except for warming (Jackson et al., 2016). In our study, a reversed interaction was mostly found for *P. perfoliatus* in both tested stressor combinations. At the systems scale, the higher temperature amplified the observed synergistic effect in our experiment. Additionally, by applying a dose–response design, we revealed that interaction patterns can be dose-dependent but are consistent once a regime shift occurred. This has not previously been demonstrated, as most studies focussed on a low–high dose design (e.g. Liu et al., 2021a) supporting the need to use gradient studies rather than replicated studies with less stressor levels or a hybrid of both, when interaction patterns of stressors need to be defined.

5. Conclusion

Our study demonstrated a clear dose-dependency of effects leading to regime shifts in shallow aquatic ecosystems above a critical threshold. We have shown that warmer temperatures amplify the strength of synergism between nutrients and pesticides at environmentally relevant concentrations supporting the relevance of multiple stressor research for ecosystem management. Synergistic interactions result in a more pronounced decrease of macrophytes than would be expected from stressor addition alone. Consequently, increased temperature reduces the critical threshold concentration of other stressors causing macrophyte decline. This indicates a higher vulnerability of the system to regime shifts and a potential reduction of the safe operating space (Scheffer et al., 2015) of shallow freshwater ecosystems exposed to agricultural run-off. The risk of regime shifts might increase under further climate change but may be mitigated by reducing nutrient and pesticide loading. Further field studies may reveal how these results upscale to more complex in-situ conditions. Our study highlights a need of a scientifically informed definition of safe operating spaces in aquatic management, and demands consideration of complex stressor interactions, indirect effects, and the sensitivity of thresholds towards confounding factors including climate change.

563 Acknowledgements

564 This work is part of the CLIMSHIFT French–German project funded by the DFG [SCHM 2555/5-1 to
565 MSJ, HI 1380/8-1 to SH and STI 180/9-1 to HS] and ANR [ANR-17-CE32-0013 to EMG & JL].
566 Further, we thank P. Wellner and S. Aulhorn for assistance during sampling and M. Krauss, H.
567 Schupke & R. Gunold for assistance with pesticide analytics. Thanks for V.Goncalves from the “Pôle
568 de compétences en chimie analytique environnementale” (LIEC – ANATELO - Université de 694
569 Lorraine – CNRS) for copper analytics. We thank Anne Schechner for linguistic refinement and
570 *Imerys* (France) for provision of Kaolin free of charge. We declare that there are no conflicts of
571 interest.

572 Authors contribution

573 The concept of the CLIMSHIFT project was developed by MSJ, EG, SH, HS, FH, JL and the
574 implementation of the experiments discussed in consortium including JA, VV, NK. The experiment
575 was planned by all contributing authors. The experiment was carried out by BP. NK provided
576 macrophytes for the experiment. FH and MSJ assisted with data assessment. The paper was written
577 by BP with major contributions by MSJ & SH. Further, all authors contributed to writing and editing
578 of the paper and numerous discussions.

579

References

Allen, J., Gross, E.M., Courcoul, C., Bouletreau, S., Compin, A., Elger, A., Ferriol, J., Hilt, S., Jassey, V.E.J., Laviale, M., Polst, B.H., Schmitt-Jansen, M., Stibor, H., Vijayaraj, V., Leflaive, J., 2021. Disentangling the direct and indirect effects of agricultural runoff on freshwater ecosystems subject to global warming: A microcosm study. Water Res. 190, 116713. https://doi.org/10.1016/j.watres.2020.116713

Barker, T., Hatton, K., O'Connor, M., Connor, L., Moss, B., 2008. Effects of nitrate load on submerged plant biomass and species richness: Results of a mesocosm experiment. Fundam. Appl. Limnol. 173, 89–100. https://doi.org/10.1127/1863-9135/2008/0173-0089

Blanck, H., 2002. A critical review of procedures and approaches used for assessing pollution-induced community tolerance (PICT) in biotic communities. Hum. Ecol. Risk Assess 8, 1003-1034. https://doi.org/10.1080/1080-700291905792

Cael, B.B., Heathcote, A.J., Seekell, D.A., 2017. The volume and mean depth of Earth's lakes. Geophys. Res. Lett. 44, 209–218. https://doi.org/10.1002/2016GL071378

Chalifour, A., Juneau, P., 2011. Temperature-dependent sensitivity of growth and photosynthesis of Scenedesmus obliquus, Navicula pelliculosa and two strains of Microcystis aeruginosa to the herbicide atrazine. Aquat. Toxicol. 103, 9–17. https://doi.org/10.1016/J.AQUATOX.2011.01.016

Christensen, M.R., Graham, M.D., Vinebrooke, R.D., Findlay, D.L., Paterson, M.J., Turner, M.A., 2006. Multiple anthropogenic stressors cause ecological surprises in boreal lakes. Glob. Chang. Biol. 12, 2316–2322. https://doi.org/10.1111/j.1365-2486.2006.01257.x

Coors, A., Kuckelkorn, J., Hammers-Wirtz, M., Strauss, T., 2006. Application of in-situ bioassays with macrophytes in aquatic mesocosm studies. Ecotoxicology 15, 583–591. https://doi.org/10.1007/s10646-006-0095-z

Côté, I.M., Darling, E.S., Brown, C.J., 2016. Interactions among ecosystem stressors and their importance in conservation. Proc. R. Soc. B Biol. Sci. 283, 20152592. https://doi.org/10.1098/rspb.2015.2592

607 Coutris, C., Merlina, G., Silvestre, J., Pinelli, E., Elger, A., 2011. Can we predict community-wide
608 effects of herbicides from toxicity tests on macrophyte species? Aquat. Toxicol. 101, 49–56.
609 https://doi.org/10.1016/j.aquatox.2010.08.017

610 Crain, C.M., Kroeker, K., Halpern, B.S., 2008. Interactive and cumulative effects of multiple human
611 stressors in marine systems. Ecol. Lett. 11, 1304–1315. https://doi.org/10.1111/j.1461-
612 0248.2008.01253.x

613 DIN_EN_26777, 1993. DIN EN 26777: Water quality determination of nitrite; molecular absorption
614 spectrometric method (ISO 6777:1984); german version EN 26777:1993.
615 https://doi.org/https://dx.doi.org/10.31030/2573097

616 DIN_EN_ISO_13395, 1996. DIN EN ISO 13395:1996-12 Water quality - Determination of nitrite
617 nitrogen and nitrate nitrogen and the sum of both by flow analysis (CFA and FIA) and
618 spectrometric detection (ISO 13395:1996); German version EN ISO 13395:1996.
619 https://doi.org/https://dx.doi.org/10.31030/7304958

620 DIN_EN_ISO_6878, 2004. DIN EN ISO 6878:2004-09 Water quality - Determination of phosphorus
621 - Ammonium molybdate spectrometric method (ISO 6878:2004); German version EN ISO
622 6878:2004. https://doi.org/https://dx.doi.org/10.31030/9552789

623 Fritz, C.O., Morris, P.E., Richler, J.J., 2012. Effect size estimates: Current use, calculations, and
624 interpretation. J. Exp. Psychol. Gen. 141, 2–18. https://doi.org/10.1037/a0024338

625 Giddings, J.M., Arts, G., Hommen, U., 2013. The relative sensitivity of macrophyte and algal species
626 to herbicides and fungicides: An analysis using species sensitivity distributions. Integr. Environ.
627 Assess. Manag. 9, 308–318. https://doi.org/10.1002/ieam.1387

628 Glass, G. V, 1976. Primary, Secondary, and Meta-Analysis of Research. Educ. Res. 5, 3–8.
629 https://doi.org/10.3102/0013189X005010003

630 Halbach, K., Möder, M., Schrader, S., Liebmann, L., Schäfer, R.B., Schneeweiss, A., Schreiner,
631 V.C., Vormeier, P., Weisner, O., Liess, M., Reemtsma, T., 2021. Small streams–large
632 concentrations? Pesticide monitoring in small agricultural streams in Germany during dry
633 weather and rainfall. Water Res. 203, 117535. https://doi.org/10.1016/J.WATRES.2021.117535

Hansson, L., Ekvall, M.K., He, L., Li, Z., Svensson, M., Urrutia-Cordero, P., Zhang, H., 2020. Different climate scenarios alter dominance patterns among aquatic primary producers in temperate systems. Limnol. Oceanogr., 65, 2328-2336. https://doi.org/10.1002/lno.11455

Hilt, S., Brothers, S., Jeppesen, E., Veraart, A.J., Kosten, S., 2017. Translating Regime Shifts in Shallow Lakes into Changes in Ecosystem Functions and Services. Bioscience, 928–936. https://doi.org/10.1093/biosci/bix106

Jackson, L.J., 2003. Macrophyte-dominated and turbid states of shallow lakes: Evidence from Alberta lakes. Ecosystems 6, 213–223. https://doi.org/10.1007/s10021-002-0001-3

Jackson, M.C., Loewen, C.J.G., Vinebrooke, R.D., Chimimba, C.T., 2016. Net effects of multiple stressors in freshwater ecosystems: a meta-analysis. Glob. Chang. Biol. 22, 180–189. https://doi.org/10.1111/gcb.13028

James, C., Fisher, J., Russell, V., Collings, S., Moss, B., 2005. Nitrate availability and hydrophyte species richness in shallow lakes. Freshw. Biol. 50, 1049–1063. https://doi.org/10.1111/J.1365-2427.2005.01375.X

Janssen, A.B.G., Hilt, S., Kosten, S., Klein, J.J.M. de, Paerl, H.W., Waal, D.B. Van de, 2021. Shifting states, shifting services: Linking regime shifts to changes in ecosystem services of shallow lakes. Freshw. Biol. 66, 1–12. https://doi.org/10.1111/FWB.13582

Jensen, H.S., Andersen, F.O., 1992. Importance of temperature, nitrate, and pH for phosphate release from aerobic sediments of four shallow, eutrophic lakes. Limnol. Oceanogr. 37, 577–589.

Jöhnk, K.D., Huisman, J., Sharples, J., Sommeijer, B., Visser, P.M., Stroom, J.M., 2008. Summer heatwaves promote blooms of harmful cyanobacteria. Glob. Chang. Biol. 14, 495–512. https://doi.org/10.1111/j.1365-2486.2007.01510.x

Kreyling, J., Schweiger, A.H., Bahn, M., Ineson, P., Migliavacca, M., Morel-Journel, T., Christiansen, J.R., Schtickzelle, N., Larsen, K.S., 2018. To replicate, or not to replicate – that is the question: how to tackle nonlinear responses in ecological experiments. Ecol. Lett. 21, 1629–1638. https://doi.org/10.1111/ELE.13134

Larras, F., Lambert, A.-S., Pesce, S., Rimet, F., Bouchez, A., Montuelle, B., 2013. The effect of temperature and a herbicide mixture on freshwater periphytic algae. Ecotoxicol. Environ. Saf. 98, 162–170. https://doi.org/10.1016/J.ECOENV.2013.09.007

Le Bagousse-Pinguet, Y., Liancourt, P., Gross, N., Straile, D., 2012. Indirect facilitation promotes macrophyte survival and growth in freshwater ecosystems threatened by eutrophication. J. Ecol. 100, 530–538. https://doi.org/10.1111/j.1365-2745.2011.01931.x

Lefrancq, M., Jadas-Hécart, A., La Jeunesse, I., Landry, D., Payraudeau, S., 2017. High frequency monitoring of pesticides in runoff water to improve understanding of their transport and environmental impacts. Sci. Total Environ. 587–588, 75–86. https://doi.org/10.1016/J.SCITOTENV.2017.02.022

Liu, Y., Aznarez, C., Jeppesen, E., He, H., Li, W., Levi, E.E., Pablo, J., Yu, P., 2021. Responses of submerged macrophytes and periphyton to warming under two nitrogen scenarios : A microcosm study. Hydrobiologia 0123456789, 1333–1346. https://doi.org/10.1007/s10750-021-04530-z

Lürling, M., Eshetu, F., Faassen, E.J., Kosten, S., Huszar, V.L.M., 2013. Comparison of cyanobacterial and green algal growth rates at different temperatures. Freshw. Biol. 58, 552–559. https://doi.org/10.1111/j.1365-2427.2012.02866.x

Mckee, D., Hatton, K., Eaton, J.W., Atkinson, D., Atherton, A., Harvey, I., Moss, B., 2002. Effects of simulated climate warming on macrophytes in freshwater microcosm communities. Aquat. Bot. 74, 71–83. https://doi.org/10.1016/S0304-3770(02)00048-7

Mooij, W.M., Janse, J.H., De Senerpont Domis, L.N., Hülsmann, S., Ibelings, B.W., 2007. Predicting the effect of climate change on temperate shallow lakes with the ecosystem model PCLake, in: Hydrobiologia. Springer, pp. 443–454. https://doi.org/10.1007/s10750-007-0600-2

Moss, B., Kosten, S., Meerhoff, M., Battarbee, R.W., Jeppesen, E., Mazzeo, N., Havens, K., Lacerot, G., Liu, Z., De Meester, L., Paerl, H.W., Scheffer, M., 2011. Allied attack: climate change and eutrophication. Inl. Waters 1, 101–105. https://doi.org/10.5268/IW-1.2.359

Nõges, P., Argillier, C., Borja, Á., Garmendia, J.M., Hanganu, J., Kodeš, V., Pletterbauer, F.,

688 Sagouis, A., Birk, S., 2016. Quantified biotic and abiotic responses to multiple stress in
689 freshwater, marine and ground waters. Sci. Total Environ. 540, 43–52.
690 https://doi.org/10.1016/J.SCITOTENV.2015.06.045

691 Odum, E.P., Finn, J.T., Franz, E.H., 1979. Perturbation Theory and the Subsidy-Stress Gradient.
692 Bioscience 29, 349–352. https://doi.org/10.2307/1307690

693 OECD, 2014. Test No. 239: Water-Sediment Myriophyllum Spicatum Toxicity Test, OECD
694 Guidelines for the Testing of Chemicals, Section 2. OECD.
695 https://doi.org/10.1787/9789264224155-en

696 Olsen, S., Cao, Y., Florencia Gutierrez, M., Brucet, S., Landkildehus, F., Lauridsen, T.L., Davidson,
697 T.A., Søndergaard, M., Jeppesen, E., Risgaard-Petersen, N., 2017. Effect of a nitrogen pulse
698 on ecosystem N processing at different temperatures: A mesocosm experiment with $^{15}NO_3^-$
699 addition. Freshw. Biol. 62, 1232–1243. https://doi.org/10.1111/fwb.12940

700 Olsen, S., Chan, F., Li, W., Zhao, S., Søndergaard, M., Jeppesen, E., 2015. Strong impact of nitrogen
701 loading on submerged macrophytes and algae: a long-term mesocosm experiment in a shallow
702 Chinese lake. Freshw. Biol. 60, 1525–1536. https://doi.org/10.1111/fwb.12585

703 Paerl, H.W., Huisman, J., 2008. Climate: Blooms like it hot. Science 320, 57-58.
704 https://doi.org/10.1126/science.1155398

705 Phillips, G., Willby, N., Moss, B., 2016. Submerged macrophyte decline in shallow lakes: What have
706 we learnt in the last forty years? Aquat. Bot. 135, 37–45.
707 https://doi.org/10.1016/J.AQUABOT.2016.04.004

708 Pirotta, E., Thomas, L., Costa, D.P., Hall, A.J., Harris, C.M., Harwood, J., Kraus, S.D., Miller, P.J.O.,
709 Moore, M.J., Photopoulou, T., Rolland, R.M., Schwacke, L., Simmons, S.E., Southall, B.L.,
710 Tyack, P.L., 2022. Understanding the combined effects of multiple stressors: A new perspective
711 on a longstanding challenge. Sci. Total Environ. 821, 153322.
712 https://doi.org/10.1016/J.SCITOTENV.2022.153322

713 R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for
714 Statistical Computing, Vienna, Austria.

715 Richardson, J., Feuchtmayr, H., Miller, C., Hunter, P.D., Maberly, S.C., Carvalho, L., 2019.
716 Response of cyanobacteria and phytoplankton abundance to warming, extreme rainfall events
717 and nutrient enrichment. Glob. Chang. Biol. 25, 3365–3380. https://doi.org/10.1111/gcb.14701

718 Rigosi, A., Carey, C.C., Ibelings, B.W., Brookes, J.D., 2014. The interaction between climate
719 warming and eutrophication to promote cyanobacteria is dependent on trophic state and varies
720 among taxa. Limnol. Oceanogr. 59, 99–114.

721 Ritz, C., Baty, F., Streibig, J.C., Gerhard, D., 2015. Dose-Response Analysis Using R. PLoS One
722 10, e0146021. https://doi.org/10.1371/JOURNAL.PONE.0146021

723 Roussel, H., Ten-Hage, L., Joachim, S., Le Cohu, R., Gauthier, L., Bonzom, J.M., 2007. A long-term
724 copper exposure on freshwater ecosystem using lotic mesocosms: Primary producer
725 community responses. Aquat. Toxicol. 81, 168–182.
726 https://doi.org/10.1016/j.aquatox.2006.12.006

727 Rücker, J., Nixdorf, B., Quiel, K., Grüneberg, B., 2019. North German Lowland Lakes Miss Ecological
728 Water Quality Standards—A Lake Type Specific Analysis. Water 2019, Vol. 11, Page 2547 11,
729 2547. https://doi.org/10.3390/W11122547

730 Sagrario, M.A.G., Jeppesen, E., Gomà, J., Søndergaard, M., Jensen, J.P., Lauridsen, T.,
731 Landkildehus, F., 2005. Does high nitrogen loading prevent clear-water conditions in shallow
732 lakes at moderately high phosphorus concentrations? Freshw. Biol. 50, 27–41.
733 https://doi.org/10.1111/J.1365-2427.2004.01290.X

734 Sawilowsky, S., 2009. New Effect Size Rules of Thumb. J. Mod. Appl. Stat. Methods 8, 26.
735 https://doi.org/10.22237/jmasm/1257035100

736 Scheffer, M., Barrett, S., Carpenter, S.R., Folke, C., Green, A.J., Holmgren, M., Hughes, T.P.,
737 Kosten, S., van de Leemput, I.A., Nepstad, D.C., van Nes, E.H., Peeters, E.T.H.M., Walker, B.,
738 2015. Creating a safe operating space for iconic ecosystems. Science (80-.). 347, 1317 LP –
739 1319. https://doi.org/10.1126/science.aaa3769

740 Scheffer, M., Hosper, S.H., Meijer, M.-L., Moss, B., Jeppesen, E., 1993. Alternative equilibria in
741 shallow lakes. Trends Ecol. Evol. 8, 275–9. https://doi.org/10.1016/0169-5347(93)90254-M

742 Schinegger, R., Trautwein, C., Melcher, A., Schmutz, S., 2012. Multiple human pressures and their
743 spatial patterns in European running waters. Water Environ. J. 26, 261–273.
744 https://doi.org/10.1111/j.1747-6593.2011.00285.x

745 Tasmin, R., Shimasaki, Y., Tsuyama, M., Qiu, X., Khalil, F., Okino, N., Yamada, N., Fukuda, S.,
746 Kang, I.-J., Oshima, Y., 2014. Elevated water temperature reduces the acute toxicity of the
747 widely used herbicide diuron to a green alga, Pseudokirchneriella subcapitata. Environ. Sci.
748 Pollut. Res. 21, 1064–1070. https://doi.org/10.1007/s11356-013-1989-y

749 Verbeek, L., Gall, A., Hillebrand, H., Striebel, M., 2018. Warming and oligotrophication cause shifts
750 in freshwater phytoplankton communities. Glob. Chang. Biol. 24, 4532–4543.
751 https://doi.org/10.1111/gcb.14337

752 Verpoorter, C., Kutser, T., Seekell, D.A., Tranvik, L.J., 2014. A global inventory of lakes based on
753 high-resolution satellite imagery. Geophys. Res. Lett. 41, 6396–6402.
754 https://doi.org/10.1002/2014GL060641

755 Vijayaraj, V., Kipferler, N., Stibor, H., Allen, J., Hölker, F., Lavaile, M., Leflaive, J., Moreira M., G.A.L.,
756 Polst, B.H., Schmitt-Jansen, M., Hilt, S., Gross, E.M., 2022a. Evaluating multiple stressor
757 effects on benthic–pelagic freshwater communities in systems of different complexity:
758 challenges in upscaling. water.

759 Vijayaraj, V., Laviale, M., Allen, J., Amoussou, N., Hilt, S., Hölker, F., Kipferler, N., Leflaive, J., López
760 Moreira M, G.A., Polst, B.H., Schmitt-Jansen, M., Stibor, H., Gross, E.M., 2022b. Multiple-
761 stressor exposure of aquatic food webs: Nitrate and warming modulate the effect of pesticides.
762 Water Res. 216, 118325. https://doi.org/10.1016/J.WATRES.2022.118325

763 Wagenhoff, A., Townsend, C.R., Phillips, N., Matthaei, C.D., 2011. Subsidy-stress and multiple-
764 stressor effects along gradients of deposited fine sediment and dissolved nutrients in a regional
765 set of streams and rivers. Freshw. Biol. 56, 1916–1936. https://doi.org/10.1111/j.1365-
766 2427.2011.02619.x

767 Wijewardene, L., Wu, N., Qu, Y., Guo, K., Messyasz, B., Lorenz, S., Riis, T., Ulrich, U., Fohrer, N.,
768 2021. Influences of pesticides, nutrients, and local environmental variables on phytoplankton

communities in lentic small water bodies in a German lowland agricultural area. Sci. Total Environ. 780, 146481. https://doi.org/10.1016/J.SCITOTENV.2021.146481

Woolway, R.I., Jennings, E., Shatwell, T., Golub, M., Pierson, D.C., Maberly, S.C., 2021. Lake heatwaves under climate change. Nat. 2021 5897842 589, 402–407. https://doi.org/10.1038/s41586-020-03119-1

Xu, Z., Zhang, X., Xie, J., Yuan, G., Tang, X., Sun, X., Yu, G., 2014. Total Nitrogen Concentrations in Surface Water of Typical Agro- and Forest Ecosystems in China, 2004-2009. PLoS One 9, e92850. https://doi.org/10.1371/JOURNAL.PONE.0092850

Zhang, P., Grutters, B.M.C., van Leeuwen, C.H.A., Xu, J., Petruzzella, A., van den Berg, R.F., Bakker, E.S., 2019. Effects of Rising Temperature on the Growth, Stoichiometry, and Palatability of Aquatic Plants. Front. Plant Sci. 9, 1947. https://doi.org/10.3389/fpls.2018.01947