The effects of agricultural stressors and climate change on regime shifts in the dominance of aquatic phototrophic communities

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Bastian H. Polst

München, den 12. Dezember 2022

We have no need of other worlds. We need mirrors.

We don't know what to do with other worlds.

A single world, our own, suffices us.

- Stanisław Lem

Summary

In agricultural landscapes macrophyte-dominated shallow aquatic systems like ponds and lakes provide important ecosystem functions and services. These ecosystems get deteriorated by high nutrient concentrations originating from agricultural run-off as they lead to a regime shift to a phytoplankton-dominated turbid state. Agricultural run-off often not only comprises growth-promoting nutrients, especially nitrate, but also growth-inhibiting pesticides. When co-occurring, these two stressors may interact in an antagonistic, synergistic, or even reversed way. Whether the co-occurrence of pesticides in agricultural run-off affects nitrate-caused regime shifts remains unclear and thus, this question is at the core of this thesis. Another factor impacting aquatic ecosystems is elevated water temperature as a consequence of climate change which potentially modifies the effects of co-occurring nutrients and pesticides. Understanding the effects of multiple stressors, namely agricultural run-off and elevated temperatures, on regime shifts from macrophyte-dominated to phytoplankton-dominated states in shallow aquatic system is the aim of this thesis.

I used experimental setups mimicking shallow aquatic lakes and a mixture representative for agricultural run-off (ARO, including nitrate, an herbicide, insecticide, fungicide and copper) to target three objectives: 1) assessing the interactive effects of combined agricultural stressors on regime shifts between primary producers, 2) analysing if the risk of regime shifts caused by multiple agricultural stressors is modified at higher temperatures, 3) validating the results obtained in objectives 1 & 2 for different scenarios reflecting possible in-field scenarios. Therefore, the effects of exposure via the sediment are clarified and the role of higher trophic levels is addressed.

Targeting the first objective, I highlight the importance of combined stressors for regime shifts: nitrate on its own did lead to a phytoplankton bloom but did not induce a regime shift. When nitrate was combined with pesticides as ARO a regime shift was observed, as phytoplankton increased and in parallel macrophytes declined. Synergistic interactions between nitrate and pesticides were found. Further, process-based modelling of these experimental results revealed that adaptation of phytoplankton communities to ARO is one mechanism contributing to ARO-induced regime shifts. My results show that stressors with opposing mechanisms can act synergistically and thereby cause a regime shift.

The role of elevated temperatures (up to +4°C) on shifts between alternative states is scope of the second objective and revealed reversing effects: While elevated temperature without pollution strengthened the macrophyte-dominate state, it increased the risk for ARO-induced regime shifts in simplified settings with phototrophic communities. In settings with higher trophic levels (grazers and filter feeders), ARO interacts in a complex way with elevated temperatures and can modify the effect of ARO to an extent that does not allow for a clear interpretation.

Targeting the third objective, validating the results for scenarios closer to field scenarios, revealed the important role of exposure pathways. ARO within the sediment impaired macrophyte growth but does lead to regime shifts as it is the case for ARO in the water phase. Further, even after increasing trophic complexity ARO still induced regime shifts. Additional factors possibly influencing the effect of ARO in the field, namely the photodegradation of pesticides and temporal dynamics of exposed stressors were identified.

In conclusion, the findings presented in my dissertation indicate a risk for ARO-induced regime shifts from macrophyte- to phytoplankton dominance in shallow aquatic systems. Changes in the microalgae and zooplankton community and related trophic interactions enhance such regime shifts. The interactions in complex communities and other environmental factors, e.g. exposure pathways and physico-chemical properties of pesticides, need to be considered when transferring these results into the field. Additionally, elevated temperatures interact with these agricultural stressors in a complex way and can further increase the risk of regime shifts. These complex interactions need to be considered when defining future-prove safe operating spaces and mitigation measures for aquatic systems in agricultural landscapes.

Zusammenfassung

In unserer landwirtschaftlich geprägten Umwelt erbringen Makrophyten-dominierte Flachgewässer wichtige Ökosystemleistungen. Hohe Nährstoffkonzentrationen, die aus landwirtschaftlichen Oberflächenabflüssen stammen, können zu einem Regimewechsel in einen Phytoplankton-dominierten, trüben Zustand führen, welcher die Leistungen des Ökosystems beeinträchtigt. Neben wachstumsfördernden Nährstoffen, insbesondere Nitrat, enthalten landwirtschaftliche Abflüsse oft auch wachstumshemmende Pestizide. Diese Stressoren können interagieren und es kann zu antagonistischen, synergistischen oder sogar reversen Interaktionen kommen, wobei die Rolle von Pestiziden auf einen nährstoffbedingten Regimewechsel noch unklar ist. Darüber hinaus wirken sich erhöhte Temperaturen, wie sie als Folge des Klimawandels auftreten, besonders auf Flachgewässer aus und können eventuell die Interaktion von Nährstoffen und Pestiziden verändern. Im Mittelpunkt dieser Doktorarbeit stand die Untersuchung der Auswirkung kombinierter Stressoren, nämlich der landwirtschaftlichen Abflüsse mit Nitrat und Pestiziden und des Klimawandels, auf den Regimewechsel in flachen aquatischen Systemen. Mittels experimenteller Modelökosysteme, die Flachgewässer nachbilden, und der Applikation einer Stoffmischung, die repräsentativ für landwirtschaftliche Abflüsse ist (ARO; bestehend aus Nitrat, einem Herbizid, Insektizid, Fungizid und Kupfer), habe ich drei Ziele verfolgt: 1) Bewerten wie sich die Interaktion der landwirtschaftlichen Stressoren auf Regimewechsel auswirkt. 2) Analyse, ob höhere Temperaturen das Risiko für solche Regimewechsel, ausgelöst durch landwirtschaftliche Abflüsse, moduliert. 3) Validierung der zuvor erzielten Ergebnisse für Szenarien, die näher an der tatsächlichen Situation im Feld liegen. Besonderer Fokus liegt hierbei auf den Expositionspfaden und der trophischen Komplexität in Flachgewässern.

Mit einem multi-faktoriellen Gradientendesign konnte ich die Bedeutung von kombinierten Stressoren für Regimewechsel erarbeiten: Während Nitrat zwar das Phytoplanktonwachstum anregte, löste Nitrat alleine keinen Regimewechsel aus. Wurde Nitrat mit Pestiziden kombiniert, trat ein solcher Regimewechsel auf: die Phytoplankton-Biomasse nimmt zu, während gleichzeitig die der Makrophyten abnimmt. Eine synergistische Interaktion von Nitrat und Pestiziden wurde festgestellt. Mittels Prozess-basierter Modellierung wurde gezeigt, dass die Anpassungsfähigkeit der Phytoplanktongemeinschaft hierbei ein wichtiger Mechanismus ist. Meine Ergebnisse zeigen, dass landwirtschaftliche Stressoren mit gegensätzlichen Mechanismen durch eine synergistische Interaktion zu einem Regimewechsel führen kann.

Die genauere Betrachtung der Rolle erhöhter Temperaturen (bis zu +4°C) ergab gegenläufige Effekte: Während erhöhte Temperaturen den Makrophyten-dominierten Zustand fördern, erhöhen sie das Risiko für einen ARO-induzierten Regimewechsel. Steigert man zusätzlich die trophische Komplexität, interagiert ARO auf komplexe Weise mit höheren Temperaturen und kann die Wirkung von ARO in einem Ausmaß verändern, die keine eindeutige Interpretation zulässt.

Die Validierung der vorherigen Ergebnisse für Szenarien, die der tatsächlichen Situation im Feld näherkommen, zeigte die wichtige Rolle der Expositionspfade. Die Exposition von ARO über das Sediment kann das Wachstum der Makrophyten beeinträchtigen, führt aber nicht zu Regimewechseln, wie es bei der Exposition über die Wasserphase der Fall ist. Darüber hinaus führte ARO auch bei zunehmender trophischer Komplexität noch zu einem Regimewechsel. Zusätzliche Faktoren, die möglicherweise die Wirkung von ARO im Freiland beeinflussen, sind die Photolyse von Pestiziden und die zeitliche Dynamik von exponierten Stressoren und deren zeitverzögerten Auswirkungen.

Insgesamt zeigen die in dieser Dissertation vorgestellten Ergebnisse ein erhöhtes Risiko von ARO-induzierten Regimewechseln von Makrophyten- zur Phytoplanktondominanz in flachen aquatischen Systemen auf. Veränderungen in der Mikroalgen- und Zooplankton-Gemeinschaft tragen zu einem solchen Regimewechsel bei. Diese komplexe Zusammensetzung der Lebensgemeinschaften und andere abiotische Faktoren, z. B. die Expositionspfade im Feld und physikalisch-chemische Eigenschaften von Pestiziden, müssen bei der Übertragung der Ergebnisse auf die Praxis berücksichtigt werden. Außerdem stehen erhöhte Temperaturen in komplexer Wechselwirkung mit diesen landwirtschaftlichen Stressfaktoren und illustrieren die Relevanz von Szenarien des Klimawandels für das Risiko von Regimewechseln in aquatischen Systemen. Diese komplizierten Interaktionen müssen berücksichtigt werden, um Grenzwerte und Maßnahmen für landwirtschaftlich beeinflusste Gewässer zu definieren, die auch in Zukunft Gültigkeit behalten.

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1. Introduction

1.1 Occurrence and relevance of shallow lakes

Shallow aquatic ecosystems such as ponds, shallow lakes, ditches and kettle holes are the most abundant types of freshwater ecosystems globally (Downing et al., 2006; Verpoorter et al., 2014; Cael et al., 2017; Kazanjian et al., 2018). There are about 90 million lakes of a size between 0.002 and 0.01 km² and probably a higher number of even smaller lakes and ponds (Verpoorter et al., 2014). The depth of such shallow aquatic systems reaches up to approximately 5 meters (Nõges et al., 2003; Richardson et al., 2022). While these small aquatic systems comprise only a minor share in global non-glacial freshwater volume, they are at the core of the continental terrestrial-freshwater interface (Verpoorter et al., 2014). They provide important ecosystem functions and services (Hilt et al., 2017; Janssen et al., 2021; Lischeid et al., 2018). Shallow aquatic ecosystems provide habitats for rich biodiversity (Williams et al., 2004), especially macrophytes and invertebrates (Hassall et al., 2011; Pätzig et al., 2012; see figure 1), and retain and remove nutrients as well as diverse pollutants (Barko and James, 1998; Céréghino et al., 2013) and act as biogeochemical hotspots (Cheng and Basu, 2017).

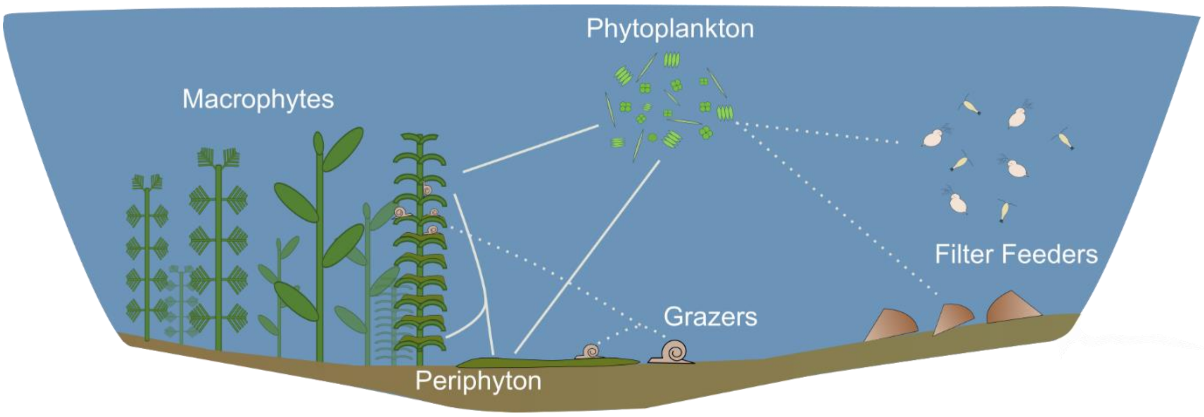

Figure 1 | Key Interactions between organism groups within fishless shallow lakes. Solid lines represent competition for light and nutrients, dotted lines represent feeding pressure (filter feeders ➔ phytoplankton, grazers ➔ periphyton & macrophytes).

These small and shallow aquatic ecosystems are particularly present in agricultural landscapes (Beklioğlu et al., 2016; Lischeid et al., 2018; Rücker et al., 2019) and are commonly fishless or only habit a poor fish community (Lancelotti et al., 2009; Scheffer et al., 2006). Due to the high volume to surface ratio shallow aquatic ecosystems have a high potential for strong primary producer dominance, most commonly dominated by macrophytes, but dominance by periphyton or phytoplankton is possible as well, depending on the environmental conditions (Scheffer & Van Nes, 2007). These phototrophic organisms experience feeding pressure respectively top-down control by filter feeders (zooplankton and mussels) and grazers (snails) (figure 1).

1.2 Alternative stable states and regime shifts in shallow lakes

Dominance by either macrophytes or phytoplankton establishes itself through self-enforcing feedback loops (figure 2; van Nes et al., 2016; Dakos et al., 2019). Primary producers compete mainly for light and nutrients. The faster growing or already dominating group limits access of these resources to the other groups. While at lower nutrient concentrations macrophytes dominate, as they can take up additional nutrients available in the sediment and produce allelopathic compounds that limit phytoplankton growth (Gross et al., 2007), at higher nutrient concentrations phytoplankton dominates and shades the other primary producers.

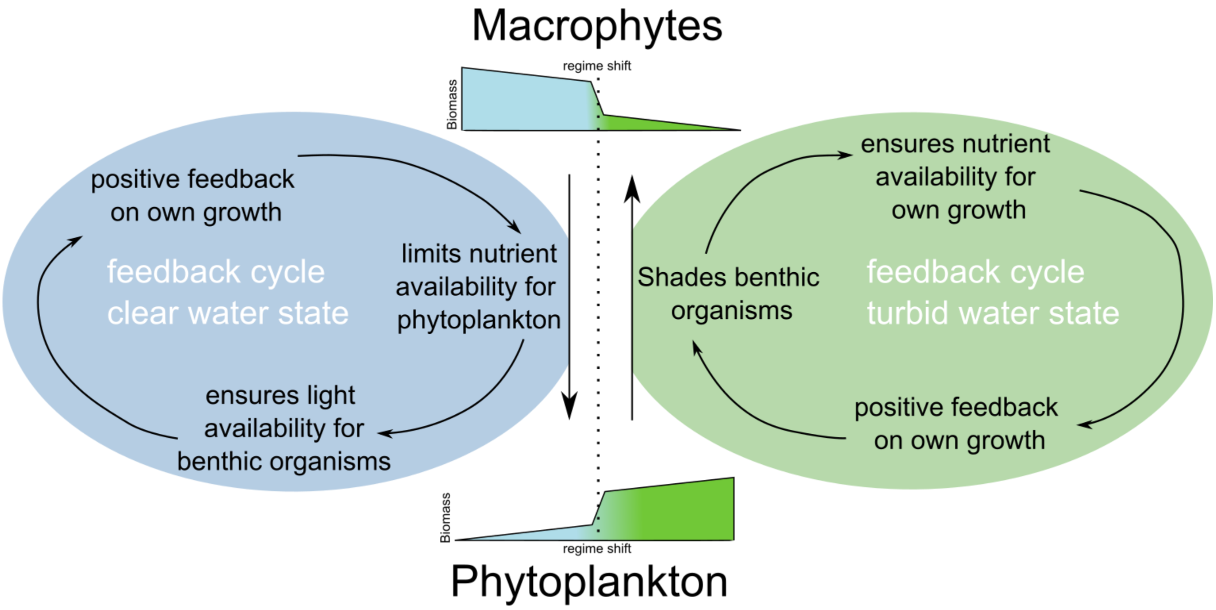

Figure 2 | The self-enforcing feedback cycles of alternative stable states. The macrophyte-dominated clear water state (blue) and a phytoplankton dominated turbid state (green) occur in dependence of the nutrient availability. The states sustain themselves through positive feedback cycles as long as a certain threshold is not exceeded. Once a threshold in nutrient availability is exceeded and phytoplankton enters its self-enhancing feedback cycle, macrophyte biomass decrease and a regime shift takes place.

Phytoplankton can make use of high nutrient concentrations faster than macrophytes due to lower doubling times respectively higher growth rates. The shift between these two states of dominance occurs rapidly when the threshold for the resilience of one state is exceeded and is referred to as a *regime shift* (Scheffer et al., 1993). Next to phytoplankton, periphyton can dominate the ecosystems by growing on macrophyte leaves (epiphyton) and therefore shading macrophytes or by growing on the sediment surface (epipsammon) and hinder macrophyte germination in the first place (Roberts et al., 2003). Yet, the relationship of macrophytes and periphyton is complex, as – in return – macrophytes can limit periphyton growth through nutrient competition and allelopathy (Erhard and Gross, 2006; Wijewardene et al., 2022), whereby the role of allelopathy on microalgae growth in the context of regime shifts is uncertain (Gross et al., 2007; Mulderij et al., 2007). A dominance of periphyton is not included in the classical definition of regime shifts of shallow lakes (Scheffer et al., 1993) but its relevance has been recognized in recent years (e.g. Hao et al., 2020; Hilt et al., 2018; Liu et al., 2021), yet the reasons behind dominance of periphyton are not fully understood (de Souza et al., 2015; Vadeboncoeur et al., 2021).

1.3 Nitrate in agricultural landscapes

Commonly, regime shifts from macrophyte- to phytoplankton-dominance are induced by high nutrient concentrations mostly originating from agricultural applications (Causse et al., 2015; Rücker et al., 2019). In agricultural landscapes nutrients (phosphate, nitrogen) enter nearby aquatic ecosystems directly through improper fertilizer application, as diffuse agricultural run-off following rain events or via subsurface drainage (Bilotta et al., 2008; Causse et al., 2015). The role of nitrate as polluter has long been hidden (Moss et al., 2013), despite nitrate being a major contributor to nutrient pollution from agriculture (Lassaletta et al., 2009; Xu et al., 2014). Consequently, nitrate concentrations up to 5.8 ± 3.2 mg L^{-1} were found in small lentic water bodies (Wijewardene et al., 2021) and nitrate concentrations up to 9 mg L^{-1} were found in shallow lakes (James et al., 2005) affected by agricultural run-off. Since small and shallow aquatic ecosystems are common in agricultural landscapes (Lischeid et al., 2018), they are particularly exposed to nitrate by agricultural run-off and thereby to nitrate-induced regime shifts (Moss et al., 2013).

1.4 Pesticides in agricultural landscapes

Next to nutrients, agricultural run-off also often transports pesticides to nearby shallow aquatic ecosystems (Liess et al., 2021), either dissolved in the run-off water or attached to suspended soils particles, depending on the physico-chemical properties of the pesticides (Adriaanse et al., 2017; Ulrich et al., 2013). Different types of pesticides and their respective mode-of-action target different organism groups resulting in a multitude of effects in these aquatic ecosystems. While herbicides are designed to affect primary producers, insecticides alter the zooplankton and macroinvertebrate community, and fungicides affect microbial activities. The ecosystem-wide response to pesticides included in agricultural run-off depends on the presence of different groups of pesticides in the agricultural run-off and the trophic levels in the respective ecosystems (Polazzo et al., 2021). Since herbicide exposure affects macrophytes less than planktonic algae species (Giddings et al., 2013), herbicides could eventually influence the occurrence of regime shifts but – as of my knowledge – no studies targeting herbicide-induced regime shifts exist. Furthermore, insecticides can support the occurrence of regime shifts by reducing filtration and feeding pressure on phytoplankton by filter feeders (e.g. Sayer et al., 2006).

1.5 Co-occurrence of nutrients and pesticides

A recent meta-analysis reports that more than two-thirds of aquatic systems undergo high nutrient loads resulting in eutrophication. Of these aquatic systems, 10-20% experience toxic stress and co-occurrence of toxic and nutrient-related stressors is found in 10-15% of cases (Nõges et al., 2016). While their study grouped all lentic ecosystems and did not break them down more deeply in different water body classes, actual exposure to co-occurring nutrient loads and pesticides in shallow aquatic ecosystems likely is even higher as these systems are particularly common in agriculturally influenced landscapes (Kazanjian et al., 2018; Lischeid & Kalettka, 2012). However, information on pesticide concentrations in small water bodies are underrepresented due to insufficient coverage of monitoring and mainly restricted to streams (Liess et al., 2021) and field studies measuring co-occurring nutrients and pesticides in shallow aquatic systems are very rare (e.g. Wijewardene et al., 2021).

1.6 Climate change and its effects on shallow lakes

Another stressor affecting aquatic systems globally is climate change, and is rapidly gaining in relevance as climate change related effects occur more often than predicted only few years ago (Pörtner et al., 2021). Climate change has various effects on shallow aquatic ecosystems, e.g. higher CO_2 concentrations in the water (Bates et al., 2008), higher fluctuations of the hydrological regime (Hayashi & van der Kamp, 2021; Jeppesen et al., 2014) and increased likelihood of drastic changes in temperature (Meerhoff et al., 2012; Woolway et al., 2021). The latter is of high relevance for shallow lakes, ponds and streams as they are rapidly heated due to their low surface-volume ratio, leading to a strong correlation with air temperatures (Woolway et al., 2016; Dokulil et al., 2021). An increase of lake water temperature by +4°C is predicted during heatwaves in the optimistic climate change scenarios, with even higher increases predicted for more dramatic climate change scenarios (Woolway et al., 2021). Next to these direct effects of climate change, a multitude of indirect effects take place, e.g. by higher surface run-off following stronger rain events, increasing concentrations of nutrients and pesticides through decreasing water volumes or changes in species interaction through different optimal temperatures (McKee et al., 2002; Marshall and Randhir, 2008; Jeppesen et al., 2011; Wu et al., 2021). Thus, higher temperatures due to climate change-related heatwaves or global warming in general interact with other stressors already present in the ecosystems.

1.7 Stressor Interactions along a subsidy-stress gradient

Phototrophic growth in aquatic ecosystems is controlled via nutrient availability (Moss et al., 2013). From an autecological perspective an increase in nutrients subsidises further growth of phototrophs, in particular the growth of macrophytes at low to intermediate nutrient concentrations. The subsidy effect on the macrophyte-dominated clear water state is finite when other phototrophic communities gain an advantage at intermediate to high nutrient concentrations. In complex systems shading of macrophytes by phytoplankton or periphyton (e.g. Barker et al., 2008) is the main mechanism responsible for declining macrophyte growth (Yu et al., 2015) (figure 3).

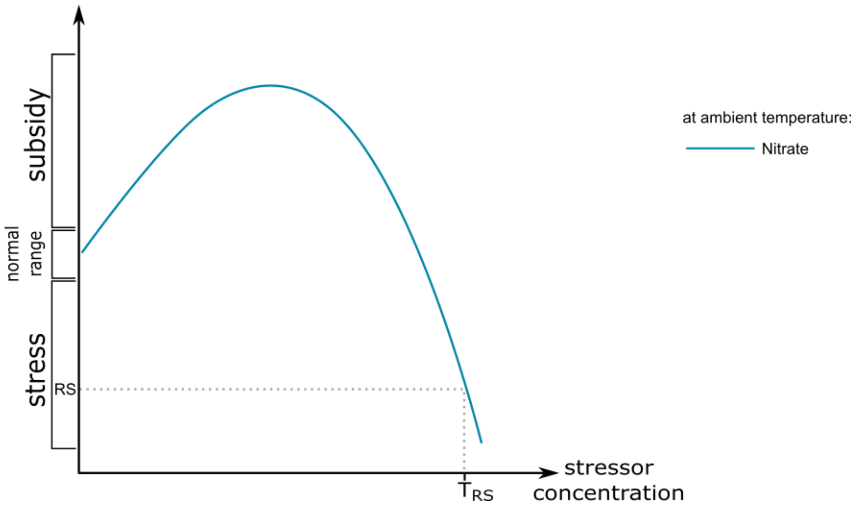

Figure 3 | The effect of nutrients in the subsidy-stress concept from the perspective of the macrophyte-dominated clear water state. Regime shifts (RS) occur once the stressor exceeds a threshold concentration (T_{RS}).

Toxins, on the other side, do not subsidise growth but exert stress on organisms even at low concentrations. Thereby, co-occurring nutrients and pesticides lead to a complex subsidy-stress trade-off and their combined effect changes along stressor gradients (Odum et al., 1979) (figure 4).

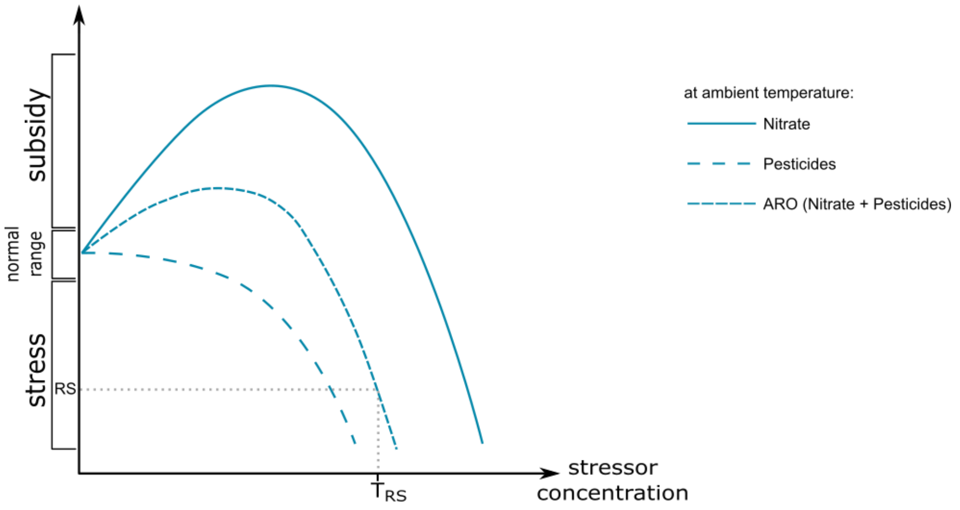

Figure 4 | The interactions of co-occurring nutrients and pesticides in the subsidy-stress concept from the perspective of the macrophyte-dominated clear water state. Pesticides do not subsidy but exert stress on the system, and potentially even modify the subsidy-stress relationship of nutrients when co-occurring in agricultural run-off (ARO). Thereby, pesticides can eventually lower the threshold for nutrient induced regime shifts (T_{RS}).

The effect of combined stressors can differ from the addition of individual effects when multiple stressors interact (figure 5). There is not yet a uniform approach to identify and classify interaction types within aquatic ecology (Orr et al., 2020). Yet, most studies follow a classification in interaction types as *antagonistic* and *synergistic* next to additive effects (Côté et al., 2016). An *additive* effect of combined stressors is observed when the individual effects of the two or more stressors add up to the observed combined effect without further interaction. An *antagonistic* interaction of combined stressors is observed, when the observed effect is between zero (no effect observed) and the sum (additive effect) of two one-directional acting or within the range of the two bi-directional acting stressors. A *synergistic* interaction is observed when the observed effect is higher than the sum of two one-directional acting stressors or outside the range of two bi-directional stressors (see figure 5).

In rare cases interacting one-directional stressors not only modify the strength of the combined effect but even change the direction of the combined effect through their interaction, e.g. when two stressors with positive effects lead to a combined negative effect. This additional interaction type was classified by Piggott et al. (2015) as “mitigating synergism” and further adapted by Jackson et al. (2016) as *reversed* interaction (figure 5).

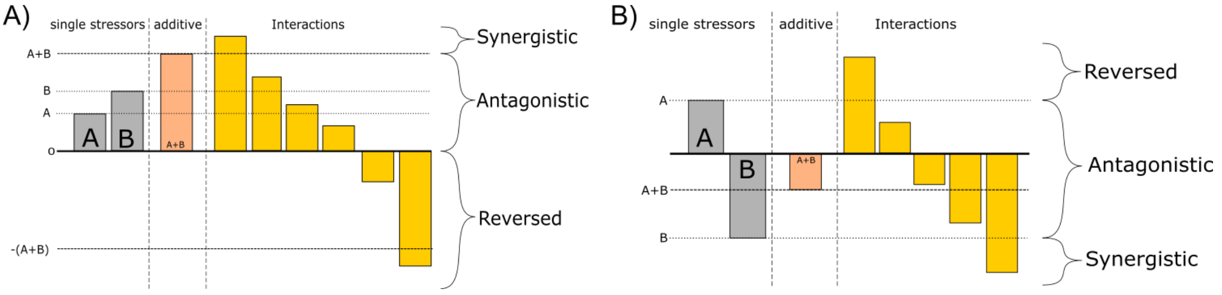

Figure 5 | Co-occurring stressors can interact in different ways. Here antagonistic, synergistic, and reversed interactions are classified based on the effect of two equidirectional (A) and oppositely (B) acting stressors in comparison with their single and their additive effect.

External factors or further additional stressors, e.g. elevated temperatures, can influence the sensitivity of aquatic systems to already occurring individual stressors and ultimately modify their combined effect respectively interaction (figure 6). While the effect of elevated temperatures and nutrients was tested (e.g. McKee et al., 2003; Moss et al., 2003; Piggott et al., 2012) as well as the effect of elevated temperatures and pesticides (e.g. Larras et al., 2013; Tasmin et al., 2014), the existing studies do not allow projection of the effect of increased temperature on this subsidy-stress combination in the context of regime shifts. Two possible scenarios for changes of the effect of combined nitrate and pesticides along the subsidy-stress gradient are shown as an example (figure 6).

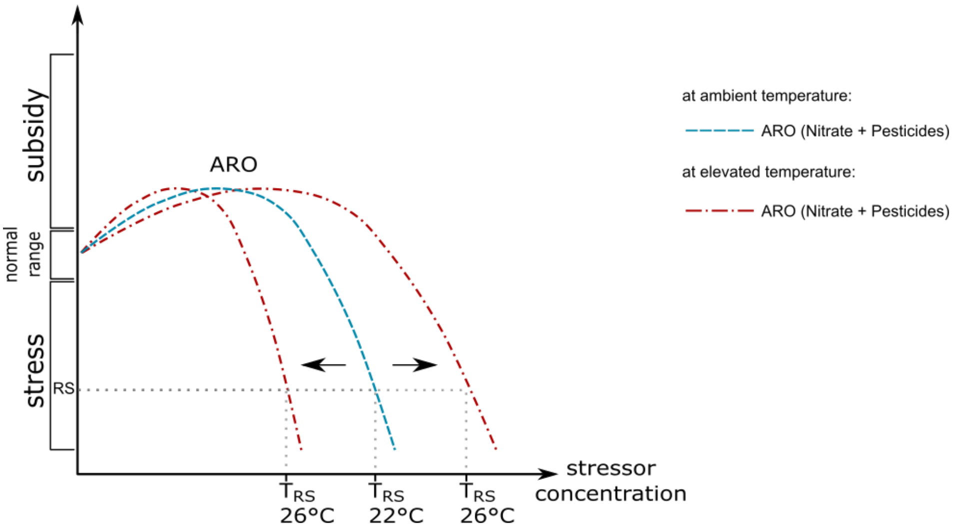

Figure 6 | The potential effect of higher temperatures on ARO in the subsidy-stress concept from the perspective of the macrophyte-dominated clear water state. As a consequence the threshold of ARO-induced regime shifts (T_{RS}) could be modified, e.g. to a higher or lower ARO concentration.

Their combined effect stays the same when the change in sensitivity to the stressors outweigh each other, it can be worsened when the sensitivity acts in the same direction, or one stressor dominates the combined effect. This way elevated temperatures might lower sensitivity to pesticides while in parallel the sensitivity to nutrients increases, leading to no change in their combined effect. When the third stressor changes sensitivity of the two other stressors in the same direction, e.g. when elevated temperature increases pesticide and nutrient sensitivity in parallel, the combined effect can be stronger than without the third stressor.

1.8 First Objective: Effects of interacting agricultural stressors on alternative stable states of shallow aquatic ecosystems

The combined effect of nutrients and pesticides has mostly been assessed in regard to single species or single species groups like periphyton, macrophytes, or phytoplankton (e.g. Murdock et al., 2013; Nuttens et al., 2016; Pannard et al., 2009; Rossi et al., 2018). The combined effect of these two stressors varies depending on actual concentrations along the subsidy-stress gradient and biological endpoints used in the studies. For example, Murdock et al. (2013)

found that high nutrient concentrations lower the effect of an herbicide on periphyton, while Nuttens et al (2016) found stronger reduction in macrophyte growth when both stressors were present. When no clear effects on biomass-related parameters are observed, changes in species competition is often hypothesised (e.g. Pannard et al., 2009). Remote and meta-studies support the overall hypothesis that agricultural land use leads to phytoplankton blooms (Kakouei et al., 2021), but often focus only on nutrient concentrations and do not account for co-occurring pesticides (Nielsen et al., 2012). Multiple stressor studies accounting for competing phototrophic groups or even interactions with higher trophic levels are rare and do not focus on ecosystem wide effects as alternative stable states respectively regime shifts (Bracewell et al., 2019; Polazzo et al., 2021). Concluding, the role of co-occurring nitrate and pesticides remains unclear even for the first trophic level (primary producers) and even more so for multiple trophic levels.

The **first objective** of this thesis is to assess the interactive effects of multiple agricultural stressors on regime shifts between primary producers.

1.9 Second objective: Interacting agricultural stressors on alternative stable states of shallow aquatic ecosystems under climate change

The effect of elevated temperature caused by climate change on alternative stable states in shallow lakes is difficult to precise. The ultimate effect of higher temperature alone is yet topic of discussion, as it can lead to macrophyte dominance (Hansson et al., 2020), or phytoplankton dominance (Paerl and Huisman, 2008) or even promote periphyton (Mahdy et al., 2015). Further, higher temperature can interact with agricultural stressors. Once again, the combined effects of higher temperature and nutrients in shallow aquatic ecosystems are complex and studies do not report consistent results. While micro- and mesocosm studies often report a promoted periphyton respectively benthic algae growth at high nutrient concentrations (Ökzan et al., 2010; Trochine et al., 2011; Hao et al., 2020), this effect depends on the response of the presence of macrophyte species present (Yang Liu et al., 2021; Wu et al., 2021). Remote and meta studies report that phytoplankton and in particular cyanobacteria blooms become more likely in nutrient rich ecosystems due to higher temperatures (Moss, 2011; Kosten et al., 2012; Beaulieu et al., 2013).

On the other hand, it was shown that higher temperatures may limit the negative effects of pesticides (Larras et al., 2013; Tasmin et al., 2014). The effect of higher temperatures on herbicide toxicity likely depends on species present and acclimation processes (Gomes and Juneau, 2017). Microalgae can adapt to herbicide pollution on a cellular level while the whole community can adapt via selection of more tolerant species within the community (Tlili et al., 2015). Yet the speed of potential adaption varies for the different microalgae groups. As a consequence of this, diatoms are at disadvantage due to their lower biovolume and consequently higher herbicide sensitivity while the community composition shifts to cyanobacteria dominance when exposed to herbicides at higher temperatures (Gomes and Juneau, 2017). Comparable studies for submerged aquatic macrophytes are missing. In conclusion, effects of higher temperature on the competition of phototrophic groups under multiple agricultural stressors cannot be projected based on current knowledge.

The **second objective** of this thesis is to analyse the risk of regime shifts caused by agricultural stressors and potential modifications by higher temperatures.

1.10 Third objective: Role of exposure pathways and trophic interactions for regime shifts induced by agricultural stressors

The main cause for increased phytoplankton growth and hence regime shifts are higher nutrient concentrations in the water column. This exposure pathway dominates in experimental settings (e.g Liu et al., 2021; Pereira et al., 2017; van Wijngaarden et al., 2005). Such studies neglect the occurrence and release of agricultural run-off via the sediment. Various studies have proven the presence of high nutrient and pesticide concentrations in the sediments of aquatic systems (Otto et al., 2016; Qu et al., 2017; Machate et al., 2021), originating from subsurface flow (Bilotta et al., 2008), bound to suspended soil particles (Ulrich et al., 2013), or absorbed from the water column (Adriaanse et al., 2022). Further, the release of nutrients and pesticides from the sediment to the water phase can be influenced by organisms (Diepens et al., 2014) and increasing temperatures (Duan and Kaushal, 2013). Whether both exposure pathways, via the sediment or the water phase, can lead to regime shifts is unclear. To clarify the role of exposure pathways and if they need further consideration when transferring experimental results to field scale, is the **first part of the third objective** of this thesis.

The competition for light and nutrients between phytoplankton, periphyton and phytoplankton is the main mechanism determining the state of the ecosystem in shallow lakes (Scheffer et al., 1993; van Nes et al., 2016). In real-world scenarios, each of these biological components is usually impacted directly by a higher trophic level. Filter feeders such as mussels and diverse zooplankton species can reduce the phytoplankton biomass (Scheffer, 1999). Snails and zooplankton graze on periphyton and even macrophytes to some part (Phillips et al., 2016; Yang et al., 2020). Higher trophic levels themselves, their interactions within these levels and their feeding pressure on lower trophic levels are impacted by multiple stressors, too (Kong et al., 2016; Griffiths et al., 2021). For example, higher temperatures modify feeding behaviour of filter feeders (Loiterton et al., 2004; Hardenbicker et al., 2015), but temperatures can easily exceed optimal levels and reach lethal temperatures during heatwaves (White et al., 2015). Similarly, pesticides decrease biological fitness and survivability, leading to higher death and lower spawning rates (Hanazato, 2001). Studies on the combined effect of agricultural run-off and climate related warming considering whole ecosystem effects are rare (e.g. Polazzo et al., 2021; Zhang et al., 2022). To clarify the role of higher trophic levels and if they need further consideration when transferring results from simplified experimental settings to field scale, is the **second part of the third objective** of this thesis.

The **third objective** of this thesis is to validate the before described results for different scenarios reflecting possible in-field scenarios. In the first part (a) the effects of exposure via the sediment are clarified, in the second part (b) the role of higher trophic levels is addressed.

1.11 Objectives of this thesis

To address the effect of multiple agricultural stressors, namely nitrate and pesticides, in perspective of climate change, on the competition of aquatic phototrophic communities, the following objectives are at the core of this thesis:

1) Assessing the interactive effects of combined agricultural stressors on regime shifts between primary producers.
2) Analysing if the risk of regime shifts caused by multiple agricultural stressors is modified at higher temperatures.
3) Validating the results obtained in objectives 1 & 2 for different scenarios reflecting possible in-field scenarios. In the first part (a) the effects of exposure via the sediment are clarified, in the second part (b) the role of higher trophic levels is addressed.

1.12 Framework of this thesis: the Climshift project

The work presented in my thesis is part of the French-German project *Climshift*, funded by the French National Research Agency ANR and the German Research Foundation DFG. The *Climshift* project aims to define safe operating spaces for shallow aquatic systems affected by agricultural stressors and climate change. Threshold for agricultural stressors should be defined based on the occurrence of regime shifts. These thresholds should hold true in the future when climate change effects, in particular elevated temperatures, become more severe. The German project partners include the Helmholtz-Centre for Environmental Research (UFZ) Leipzig, the Leibniz Institute for Freshwater Ecology and Inland Fisheries (IGB) Berlin and the Ludwig-Maximilians-University Munich. The French project partners include the Laboratoire Ecologie Fonctionnelle et Environnement (ECOLAB) Toulouse and the Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC) Metz.

1.13 Structure of this thesis

Within the following seven chapters, the objectives of this thesis will be addressed:

In chapter 2 **Material & Methods**, the setup of the microcosm and mesocosm systems used as model ecosystems in this thesis are explained. Further, the mixture representing agricultural run-off is introduced. Ultimately, an overview of the experimental designs regarding ARO concentrations, and the use of elevated temperature is given.

In chapter 3 **Stressor Interactions** (Polst et al., 2022b**:** "Warming lowers critical thresholds for multiple stressor–induced shifts between aquatic primary producers"; published in *Science of the Total Environment*) the interactions of different agricultural stressors are tested along a gradient using a factorial design. Dose-response curves are modelled and give insights on the modification of regime shift thresholds. Further, the potentially modifying effect of elevated temperature on ARO-induced regime shifts is tested.

In chapter 4 **Process-based Modeling** (López Moreira M. et al., 2022.: "Microcosm experiments combined with process-based modelling reveal differential response and adaptation of aquatic primary producers to warming and agricultural run-off"; submitted to *Frontiers in Plant Science*), a process-based model is developed based on the results of a microcosm experiment. The model gives insights into processes contributing to ARO-induced regime shifts and respects the influence of elevated temperature on pesticide toxicity. This work was conducted in close cooperation with G. Mazacote at the Institute for Freshwater Ecology and Fisheries (IGB) Berlin.

In chapter 5 **Exposure Pathways** (Polst et al., 2022a: "Exposure pathways matter: Aquatic phototrophic communities respond differently to agricultural run-off released via sediment or water"; submitted to the *Journal of Applied Ecology* and currently in review), two exposure pathways, via the sediment and via the water column, of the ARO mixture were tested. The role of elevated temperature on the two pathways and its potential implications for the occurrence of regime shifts were investigated. This work was performed in cooperation with J. Allen from the Université de Lorraine respectively Université de Toulouse.

In chapter 6 **Trophic Complexity** (Vijayaraj et al., 2022a: " Evaluating multiple stressor effects on benthic–pelagic freshwater communities in systems of different complexity: challenges in upscaling"; published in *Water*), the potential of ARO-induced regime shifts was tested at different trophic complexities. In the first experiment, the established microcosms were complemented with key species of the next higher trophic level (filter feeders & grazers). In the second experiment, outdoor mesocosms with natural phyto- and zooplankton communities were constructed and the effects of ARO were tested along a gradient at two temperature regimes (ambient and heated). This work was supported by every member of the *Climshift* project (see 1.13), with significant contributions from V. Vijayaraj and N. Kipferler. The microcosm experiment was carried out at the Université de Lorraine in Metz (France), the mesocosm experiment was carried out at the Ludwig-Maximilians-University Munich (Germany).

In chapter 7 **Discussion**, the results presented in the preceding chapters are discussed in the context of the three objectives. Comparison of the conduced experiments to each other and to relevant literature.

In chapter 8 **Conclusion**, I present a conclusive perspective on the recovery of ARO-induced regime shifts, the consideration of the presented stressors in risk assessment and measures to prevent ARO-induced regime shifts.

1.14 Joint Methods

Targeting the main question of competition between phototrophic groups, which is at the core of regime shifts, I focus on those compartments in the first three chapters of this thesis. A microcosm system with macrophytes, periphyton and phytoplankton excluding higher trophic levels was used to focus on phototrophic organisms. To answer the second part of the third objective, how these formerly obtained results from simplified communities, restricted to photoautotrophs, transfer to systems with higher trophic levels, consumers (snails, mussels, zooplankton) were added to the established microcosm design as well as constructed larger outdoor mesocosms.

1.14.1 Microcosm and mesocosm setups

The design of the microcosms, which are shown in figure 7 and 8, was based on the standardised test system *No. 239: Water-Sediment Myriophyllum Spicatum Toxicity Test* (OECD, 2014). Sediment was prepared according to the protocol including quartz sand, peat, kaolin, nettle powder and CaCO3. To provide constant and comparable conditions between the individual microcosms and the different experiments, Volvic® mineral water (8 L, Danone Deutschland GmbH, Germany) was used as an aqueous medium free of pesticides and low on nutrients. Vertical strips of plastic (*Stressor Interaction, Process-based Modelling* & *Trophic Complexity* chapters 3, 4, 6) or glass (*Exposure Pathways* chapter 5) were used as surface for periphyton colonisation. An aeration system was added to provide movement of the water as it usually occurs through wind in ecosystems. The lower part of the microcosms was wrapped in dark foil to prevent an artificially increased lateral light influx. Building up on this test system, three submerged macrophyte species, typically found in shallow eutrophic ecosystems were selected (Hilt et al., 2018): *Myriophyllum spicatum*, *Potamogeton perfoliatus*, and *Elodea nuttallii*. In the *Stressor Interaction* and the *Process-based Modeling* chapters 3 & 4, selected algae species were used as inoculum for planktonic and benthic algae. In the *Exposure Pathways* chapter 4 and the replicated experiment of the *Stressor Interaction* chapter 3 a natural algae inoculum was used to increase species diversity. In the microcosm experiment of the *Trophic Complexity* chapter 6, an additional trophic level was included with filter feeding zooplankton (*Daphnia magna*) and mussels (*Dreissena polymorpha*) as well as grazing snails (*Lymnea stagnalis*) (figure 13).

Figure 7 | Microcosms before the start of the *Exposure Pathway* experiment. The strips for periphyton colonisation and wrapping of the lower part were not yet included.

Figure 8 | Microcosm at the end of the Stressor Interaction experiment.

In the *Trophic Complexity* chapter 6, the microcosm system, comprising two trophic levels, was compared with the outdoor mesocosm system also including a more complex second trophic level. The main differences between this microcosm and the mesocosm systems were the sediment, type and volume of water, the species inoculum used and exposure to external factors which could not be controlled in the outdoor mesocosm. These mesocosms, shown in figure 9, were open to external influences such as precipitation and evaporation, natural light, and invasion of additional species. Instead of the standardized sediment based on the OECD guideline, a mixture of soil and sand was used. Local well water low on nutrients was used to fill up the mesocosms (570 L). Next to the snail and mussel species from the microcosm experiment, natural zoo- and phytoplankton communities as well as natural periphyton communities were used as inoculum.

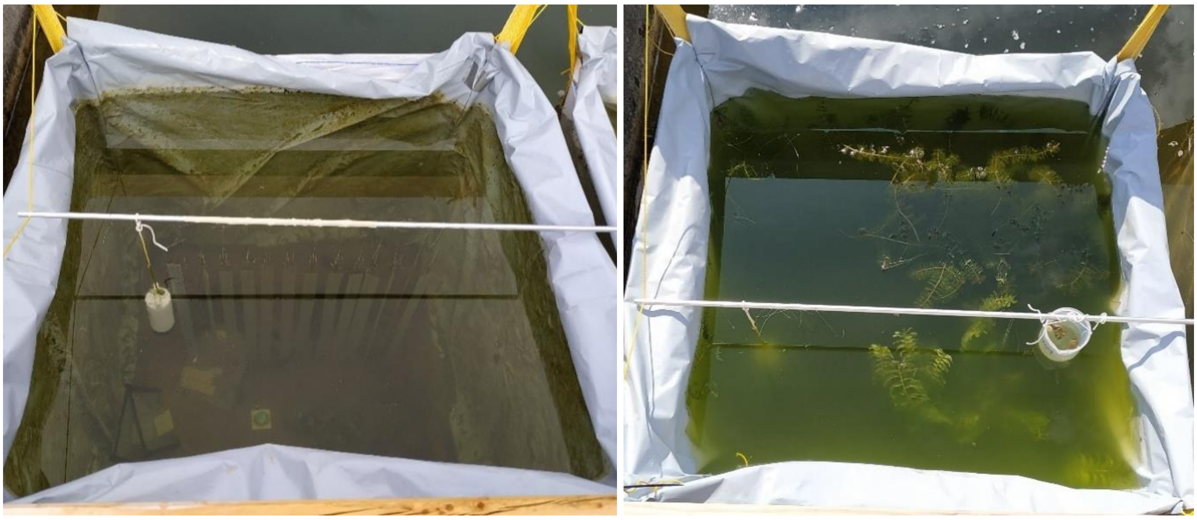

Figure 9 | Mesocosm at the start (left) and at the end (right) of the experiment in the *Trophic Complexity* chapter 6.

1.14.2 Agricultural run-off mixture (ARO)

An artificial mixture representing agricultural run-off was designed and used in all chapters/experiments. The mixture consisted of three organic pesticides, copper, which is used in inorganic farming, and nitrate (table 1). As representatives of several categories and modes of action of pesticides, terbuthylazine (herbicide, photosynthesis inhibitor), pirimicarb (insecticide, acetylcholinesterase inhibitor), tebuconazole (fungicide, dimethylase inhibitor), copper as $CuSO_4$ (algaecide & fungicide, multiple mode of action) were selected based on recent publications (Fawaz et al., 2018; Halbach et al., 2021; Liess et al., 2021; Wijewardene et al., 2021). Using single species tests and literature data, pesticide concentrations were selected in the approximate concentration of the EC20 (effective concentration at which 20% of organisms show a negative effect for the measured parameter). The mixture and the respective concentrations were published in Allen et al. (2021) for the first time:

Table 1 | Compounds, stressor type and concentration of the original ARO mixture.

	N-NO3	Terbuthylazine	Pirimicarb	Tebuconazole	Copper
Stressor	Nutrient	Herbicide	Insecticide	Fungicide	Pesticide
ARO	9000 µg L^{-1}	3 µg L^{-1}	15 µg L^{-1}	90 µg L^{-1}	42 µg L^{-1}

All three organic pesticides were recently found in agriculturally impacted ponds (Wijewardene et al., 2021). In more detail, literature reports terbuthylazine concentrations in streams and ponds ranging from 1.2 µg L^{-1} (Knauer, 2016) up to 9.6 µg L^{-1} (Baillie, 2016). For the ARO mixture a concentration of 3 µg L^{-1} terbuthylazine was selected based on the single species algae test and fits within in range of environmental concentrations reported in literature. A concentration of 15 µg L^{-1} was selected for pirimicarb based on a single species daphnia test. Data on environmental concentrations of pirimicarb are rare, and range between 0.02 µg L^{-1} (Wijewardene et al., 2021) and 2 µg L^{-1} (Kreuger, 1998). The concentration of the fungicide tebuconazole was selected based on literature data. Zubrod et al. (2011) reported a lowest observed effect concentration of 61.3 µg L^{-1} on fungal biomass associated to leaf litter disks. Artigas et al. (2012) found negative effects on periphyton functions such as lower photosynthetic activity at a concentration of 20 µg L^{-1}. In another study Maltby et al. (2009) reported a median HC5 (5 percent quantile) for non-fungal species of 238 µg L^{-1}. Due to the lack of suitable literature and the lack of standardized tests for aquatic fungi, a concentration of 90 µg L^{-1}, slightly higher than the one reported by Zubrod et al. (2011), was chosen for the ARO mixture. Copper concentrations were based on recent publication by Fawaz et al. (2018), who reported a 96 h EC50 of 65.93 µg L^{-1} for algae. For nitrate a concentration of 9 mg L^{-1} was selected based on James et al. (2005) who showed a clear decrease in submerged macrophyte species at such high nitrate concentrations.

This mixture representing agricultural run-off was the starting point for all experiments. Based on the aims and hypotheses of each experiment the ARO mixture was used in a dose-dependent design at lower or higher concentrations, but the original concentration was tested in all experiments (see table 2). In the individual experiments the different concentrations were named according to their relative enrichment factor starting at the lowest concentrations (ARO 1) respectively. This led to different naming of the same concentrations in the different experiments. For a better overview and direct comparison of ARO concentrations used in each experiment see figures 10 to 13 and table 2.

1.14.3 Experimental setup

In the first experiment – the *Stressor Interaction* chapter 3 – I split up the ARO mixture and tested individual and combined stressors in a factorial dose-response design (figure 10). In the first part, I tested the full ARO mixture in a replicated experiment to account for reproducibility of the microcosms. In the second part, I split up the ARO mixture and compared the effects of individual stressors with the full ARO mixture along a gradient. Only the full ARO mixture was tested at ambient and heated conditions. In the following, I refer to this experiment respectively the chapter as the *Stressor Interaction* chapter 3. For the next experiment I used an ARO gradient with replicated microcosms to collect robust data that were then used in a process-based model (figure 11). This model allows us to further investigate potential mechanisms relevant in regime shifts. Therefore, I refer to this work as the *Process-based Modeling* chapter 4. In the third experiment I have tested ARO exposure via two pathways, via the water column and via the sediment (figure 12). Two ARO concentrations (ARO 1 and ARO 4) were used next to a control and tested at ambient (22°C) and elevated temperature (26°C). This work is referred to as the *Exposure Pathways* chapter 5. In the last experiment, higher trophic systems were tested (figure 14). In the microcosm experiment representative key species of the first consumer level (Daphnia, snails, mussels) were used. Further, this experiment compared the Volvic® water already used in the other experiments with the well water used in the mesocosm study. In the mesocosm experiment a natural zooplankton community was used and the outdoor placement of the mesocosm allowed further immigration of other invertebrate species. Instead of a single replicated ARO treatment as used for the microcosms, the mesocosm experiment used a gradient of ARO. Both experimental designs were mirrored at ambient and heated conditions. This work is summarized in the *Trophic Complexity* chapter 6.

In each experiment, the effect of ARO was tested at ambient and elevated temperatures. For the microcosm experiments an ambient temperature of 22°C was selected as representative ambient lake water temperature during summer (Mckee et al., 2002; Sand-Jensen et al., 2019). An increase of +4°C for the elevated temperature treatments was targeted based on Woolway et. al (2021), who predict such an increase of lake water temperature during heatwaves. Since the mesocosm experiment was conducted outdoors, the ambient water temperature could not be set to a fixed temperature and was determined by outdoor factors, e.g. radiation and air temperature. For the heated mesocosm an increased temperature by +4°C in comparison to the ambient temperature was targeted, the same temperature difference attempted to achieve in the microcosm experiments.

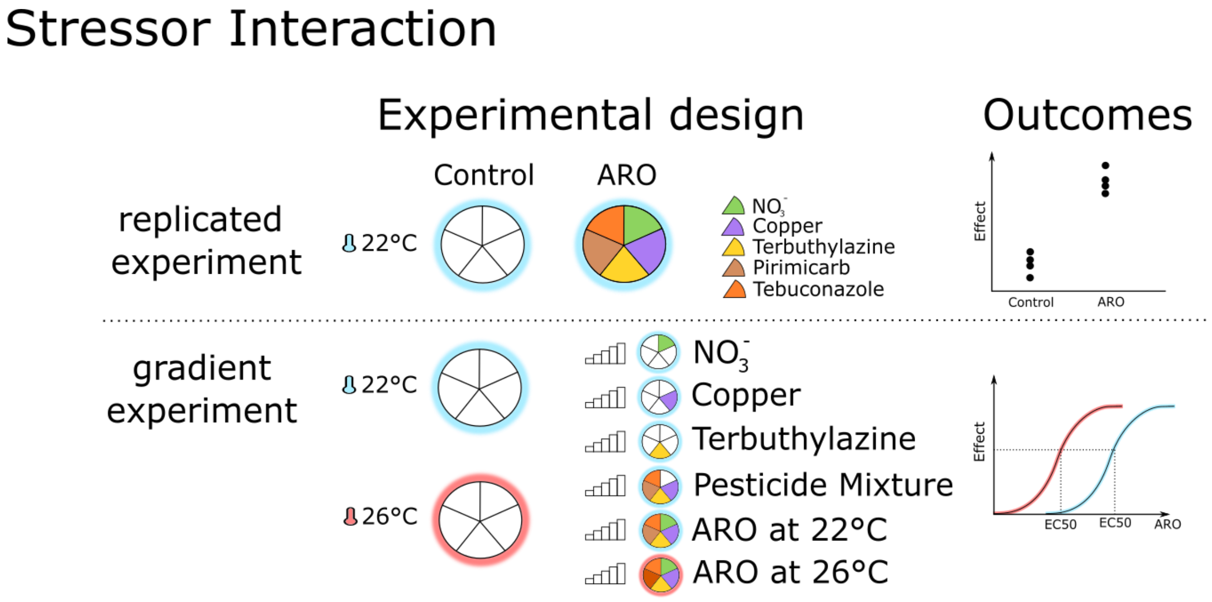

Figure 10 | The study design used in the *Stressor Interaction* chapter 3 of this thesis.

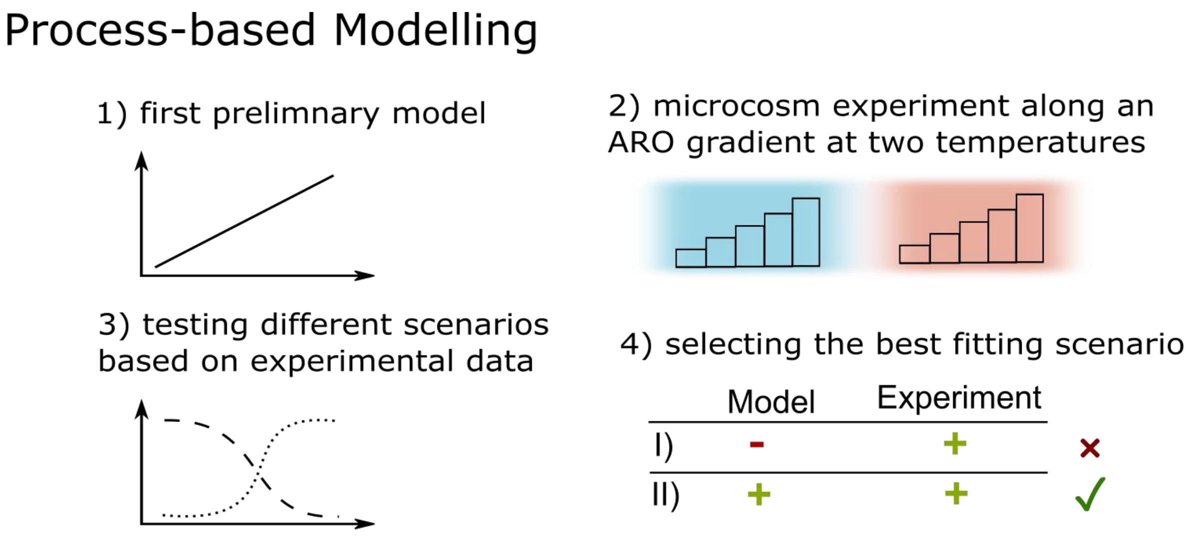

Figure 11 | The study design used in the *Process-based Modelling* chapter 4 of this thesis.

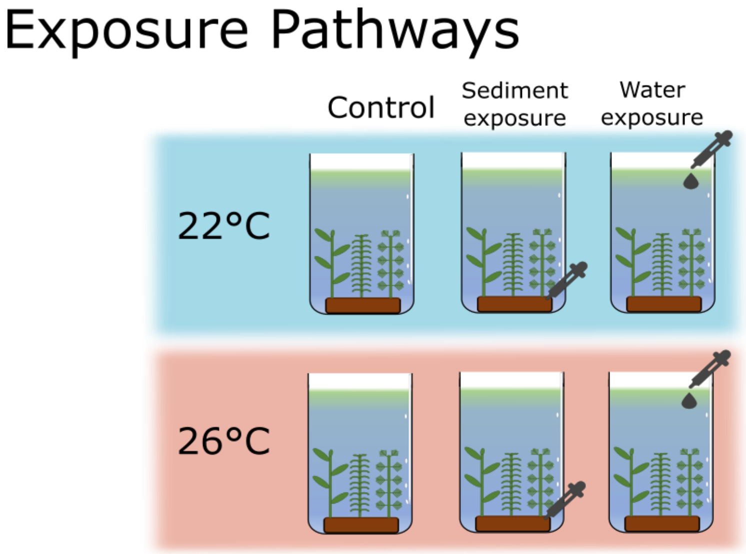

Figure 12 | The study design used in the *Exposure Pathways* chapter 5 of this thesis.

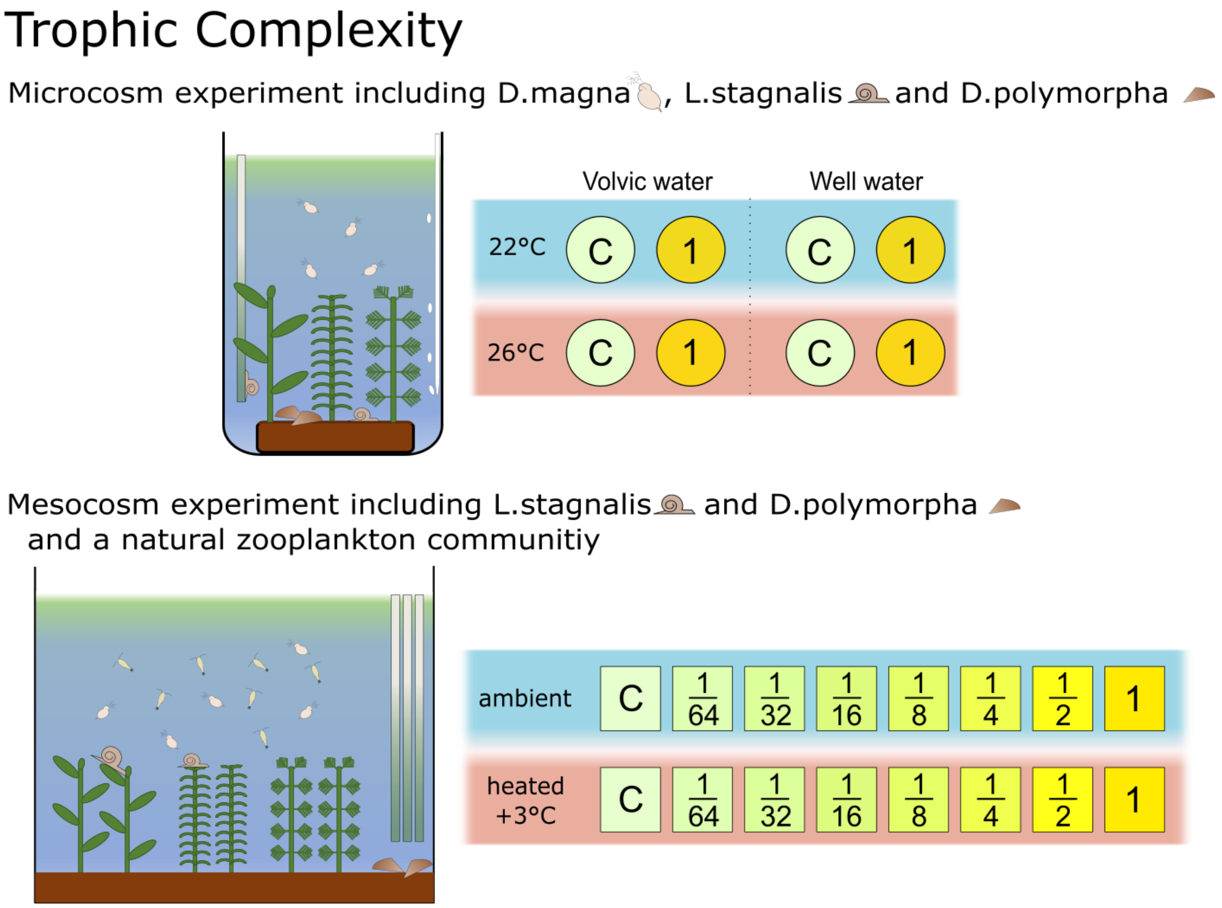

Figure 13 | The study design used in the *Trophic Complexity* chapter 6 of this thesis.

Table 2 | Concentrations and labelling of the ARO mixture in the different experiments. Concentrations were labelled according to the relative enrichment factor of the mixture for the microcosm experiments, starting with 1 for the lowest concentration used and ascending further. For the mesocosm experiment in the *Trophic Complexity* chapter 6 relative dilution factors were used, starting with 1 for the highest concentration and then descending to 1/64th of the original concentration (not all steps shown). The original ARO mixture (as shown in table 1) is marked in bold numbers. (*) For the microcosm experiment of the *Trophic Complexity* chapter 6, only one ARO concentration was used which was not further numbered. Treatments without ARO were labelled as control (C).

Chapters	0	x 1/64	…	x 1/8	x 1/4	x 1/2	**1**	x 2	x 4
Stressor Interactions (chapter 3)	C			1	2	4	**8**	16	
Process-based Modelling (chapter 4)	C				1	2	**4**	8	16
Exposure Pathways (chapter 5)	C				1		**4**		
Trophic Complexity (chapter 6) – Microcosm	C						**1***		
– Mesocosm	C	0.015	…	0.125	0.25	0.5	**1**		

2. Warming lowers critical thresholds for multiple stressor-induced regime shifts between aquatic primary producers

Bastian H. Polst, Sabine Hilt, Herwig Stibor, Franz Hölker, Joey Allen, Vinita Vijayaraj, Nora Kipferler, Jopséphine Leflaive, Elisabeth M. Gross, Mechthild Schmitt-Jansen

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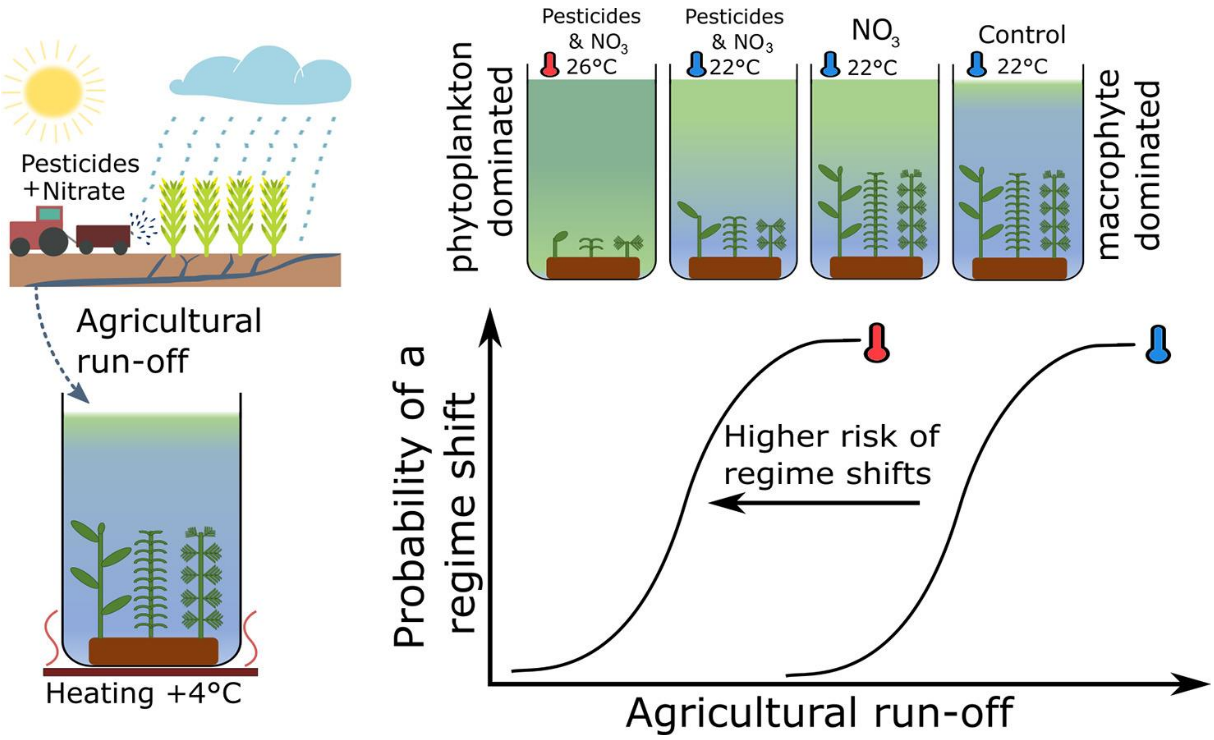
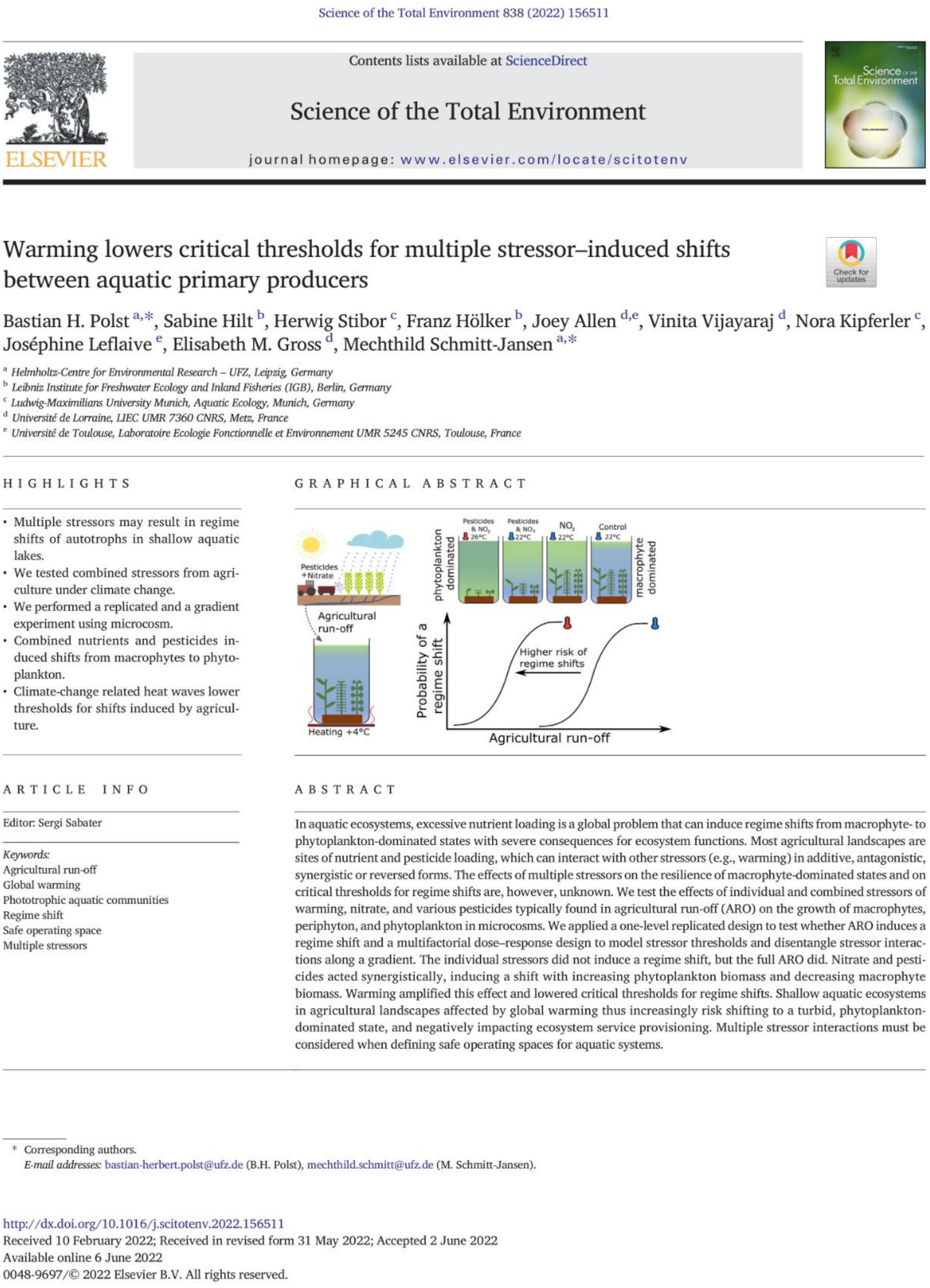


Figure 14 | Graphical abstract of the following *Stressor Interaction* chapter 3.



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

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HIGHLIGHTS

- Multiple stressors may result in regime shifts of autotrophs in shallow aquatic lakes.
- We tested combined stressors from agriculture under climate change.
- We performed a replicated and a gradient experiment using microcosm.
- Combined nutrients and pesticides induced shifts from macrophytes to phytoplankton.
- Climate-change related heat waves lower thresholds for shifts induced by agriculture.

GRAPHICAL ABSTRACT

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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.

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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 (8 L) 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 (Fig. 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 1×10^6 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 (0.5 × stock solution of WC medium) Volvic© mineral water (Danone Waters Deutschland GmbH, Germany) and were mixed in equal shares, amounting to 1×10^6 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.

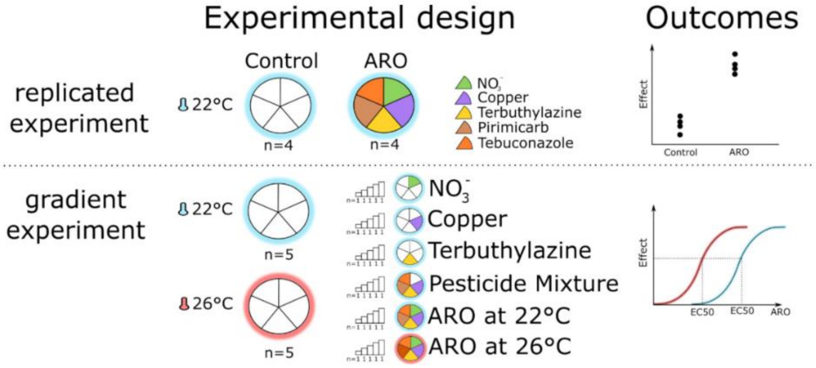

Fig. 1. Design of our study: The replicated experiment tested a control ($n = 4$) and one treatment level of agricultural run-off (ARO) (n = 4) containing nutrients and a mixture of pesticides (copper, terbuthylazine, pirimicarb, tebuconazole). The gradient experiment used two treatments at 22 °C (control, $n = 5$) 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.

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 16:8 h 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 Fig. 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 Fig. 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).

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 Fig. 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:

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

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 ($\Delta = -1$). 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

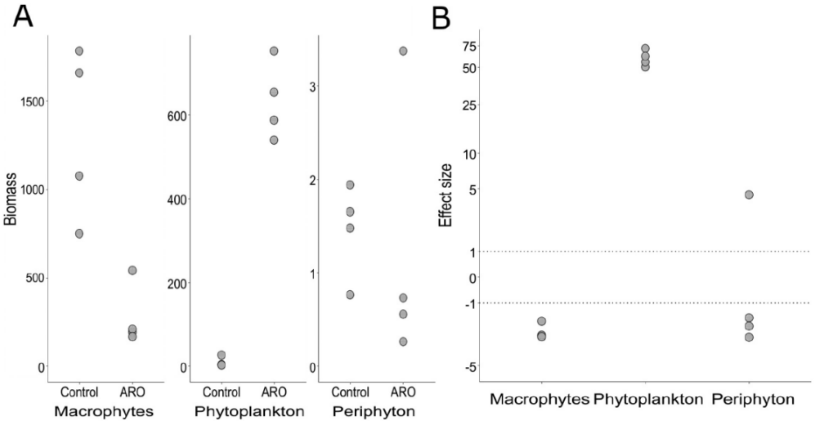

Fig. 2. A) Biomasses for macrophytes (dry weight in mg), phytoplankton (chl a in μg L^{-1}) and periphyton (dry weight in g m^{-2}) and B) Effect sizes (Glass's delta) for macrophytes (dry weight), phytoplankton (chl a) and periphyton (dry weight) after exposure to agricultural run-off for 4 weeks in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

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 Fig. 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 Fig. 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 $\mu\text{mol s}^{-1}\text{ m}^{-2}$ resp. ~30 % of surface light at day 15) compared to the ARO treatment (e.g. 8 $\mu\text{mol s}^{-1}\text{ m}^{-2}$ 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) ($p < 0.001$, Fig. 2). Their effect size averaged at −2.5 ± 0.4. Phytoplankton showed a significant increase in the ARO treatment (633 ± 80 $\mu\text{g L}^{-1}$ chl *a*) compared to the controls (9 ± 10.5 $\mu\text{g L}^{-1}$ chl *a*) with an effect size up to 75 ($p < 0.001$, Fig. 2). No significant unidirectional response of periphyton was observed. Periphyton effect size values ranged from −2.8 to 4.4 (Fig. 2), showing clear effects (effect size > 1 resp. $\Delta < -1$) in the individual microcosms but not when averaged across the replicates (−0.5 ± 3.3). A clear shift from macrophyte to phytoplankton dominance was observed in all microcosms.

3.3. Gradient experiment

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

The final macrophyte aboveground biomass showed no clear trend for the individual stressors or for the combined pesticide treatment (Fig. 3). However, individual species responded differentially, especially *P. perfoliatus* showed trends for various stressor treatments (see SI Fig. 4). Only the nitrate treatment resulted partly in negative effects on the accumulated macrophyte biomass (Fig. 4 & SI Fig. 4): While the highest nitrate concentration leads to a significant effect according to the *t*-test but not with regard to the effect sizes, the assessment of a shift for this concentration remains unclear. However, no meaningful EC50 for the final aboveground macrophyte biomass could be modelled using a log-logistic model for any of the individual stressors or the combined pesticides 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 (Fig. 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); Fig. 4). Periphyton biomass showed changes but no clear trends due to the high variability in the control samples (see SI Fig. 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 (Fig. 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 (Fig. 3). The EC50-value derived from the modelling approach of the accumulated macrophyte biomass in the ARO treatment (7.3 ± 2.7 REF, SE, Fig. 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, Fig. 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.

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 (Fig. 3). Diverse effects were observed for individual macrophyte species, e.g. only *P. perfoliatus* responded positively to warming (see SI Fig. 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 (Fig. 3). The EC50-value for the accumulated macrophyte biomass shifted towards a lower concentration (2.7 ± 0.3 REF, SE, Fig. 4) in comparison to effect values for the ARO treatment without warming (7.3 ± 2.7 REF, SE, Fig. 4). The same shift was observed for phytoplankton EC50-values (Temp + ARO: 3.9 ± 0.3 REF; ARO: 11.0 ± 4.1 REF, SE, Fig. 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 Fig. 4). Ultimately, a shift was found for the third to the highest ARO exposure concentrations.

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 (Fig. 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 (Fig. 3, see SI Table 4). There was a

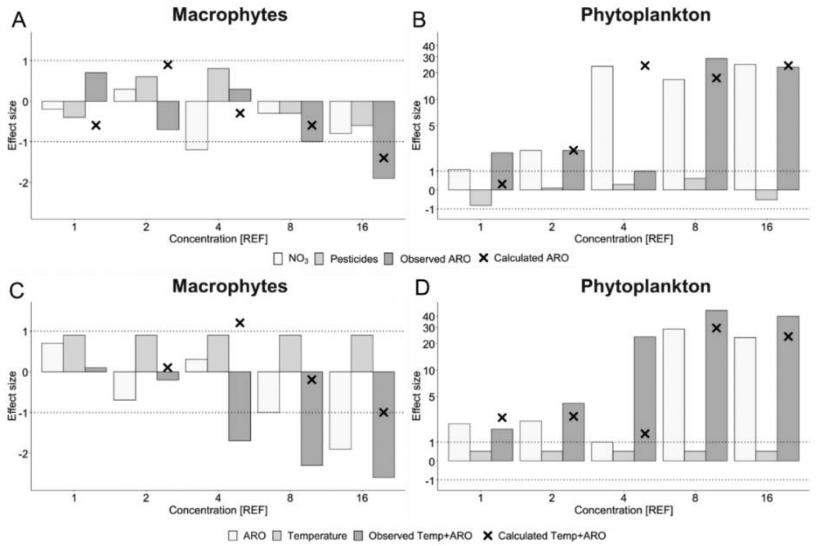

Fig. 3. Effect sizes (Glass's delta) at the end of the experiment for macrophytes (A & C) and of the phytoplankton biomass during its peak in the second week of the experiment (B & D). The response to the nitrate (NO_3), the combined pesticide treatment (Pesticides), their calculated additive effect (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).

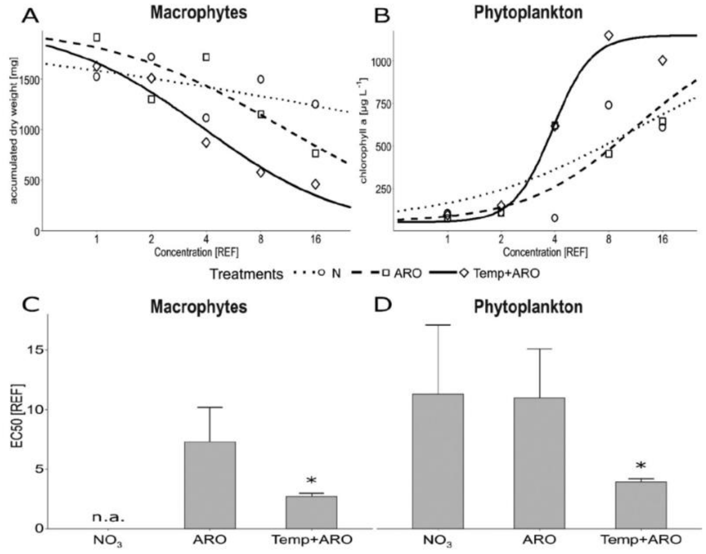

Fig. 4. Dose Response curves modelled based on the biomass data for A) macrophytes (final dry weight) and B) phytoplankton (peak chl *a*) as well as their respective EC50 values C) for macrophytes and D) for phytoplankton. EC50-values (± standard error) derived from log-logistic modelling of the gradient 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.).

remarkable response at the third concentration (C4, Fig. 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 (Fig. 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 (Fig. 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 $\mu g\ L^{-1}$ terbuthylazine. Coutris et al. (2011) also showed that several macrophyte species (including *M. spicatum*) tolerate a herbicide mixture at concentrations of 6 $\mu g\ L^{-1}$, similar to those used in our study, and only decreased in biomass at concentrations as high as 60 $\mu g\ L^{-1}$. 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 $\mu g\ L^{-1}$) 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 $\geq$ 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 effect of elevated temperature alone on phytoplankton biomass was observed in our and other studies, Allen et al. (2021) and Hansson et al. (2020) found a change in phytoplankton diversity, indicating a possible adaptation of the community to higher water temperature.

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

4.4. Elevated temperature changes threshold concentrations of ARO for regime shifts

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

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.

4.6. 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., 2021) 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.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CRediT authorship contribution statement

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.156511.

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3. Microcosm experiments combined with process-based modelling reveal differential response and adaptation of aquatic primary producers to warming and agricultural run-off

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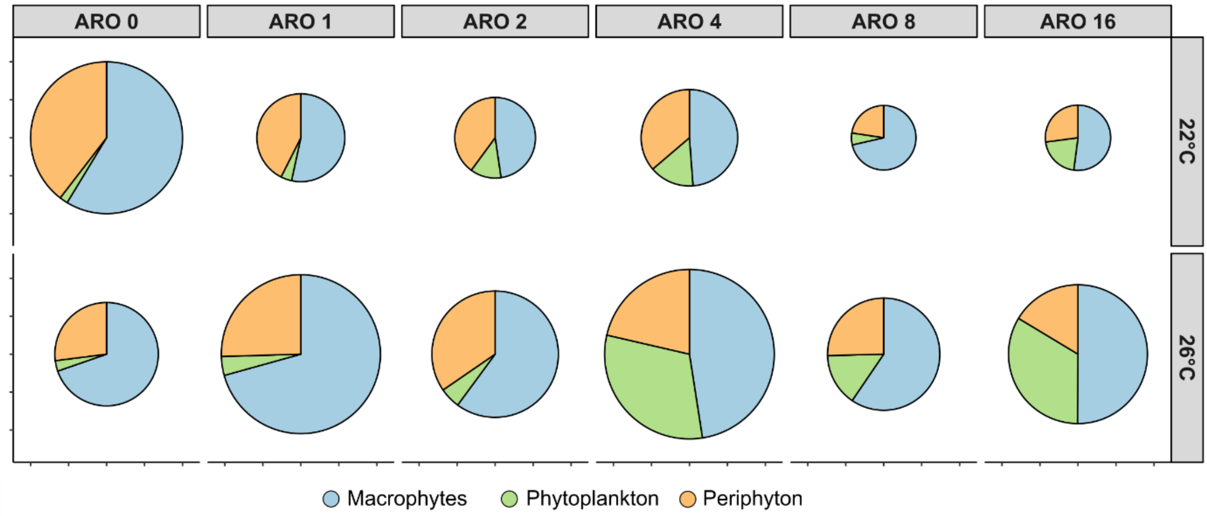

Figure 15 | Key result of the microcosm experiment of the Process-based Modelling paper: Relative accumulated biomass (size) and share of the phototrophic communities for each treatment.

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Microcosm experiments combined with process-based modeling reveal differential response and adaptation of aquatic primary producers to warming and agricultural run-off

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Abstract

Fertilizers, pesticides and global warming are threatening freshwater aquatic ecosystems. Most of these are shallow ponds or slow-flowing streams or ditches dominated by submerged macrophytes, periphyton or phytoplankton. Regime shifts between the dominance of these primary producers can occur along a gradient of nutrient loading, possibly triggered by specific disturbances interfering with their competitive interactions. However, phytoplankton dominance is less desirable due to lower biodiversity and poorer ecosystem function and services. In this study, we combined a microcosm experiment with a process-based model to test three hypotheses: 1) agricultural run-off (ARO), consisting of nitrate and a mixture of organic pesticides and copper, differentially affects primary producers and enhances the risk of regime shifts, 2) warming increases the risk of an ARO-induced regime shift to phytoplankton dominance and 3) custom-tailored process-based models support mechanistic understanding of experimental results through scenario comparison. Experimentally exposing primary producers to a gradient of nitrate and pesticides at 22°C and 26°C supported the first two hypotheses. ARO had direct negative effects on macrophytes, while phytoplankton gained from warming and indirect effects of ARO like a reduction in the competitive pressure exerted by other groups. We used the process-based model to test eight different scenarios. The best qualitative fit between modeled and observed responses was reached only when taking community adaptation and organism acclimation into account. Our results highlight the importance of considering such processes when attempting to predict the effects of multiple stressors on natural ecosystems.

1 Introduction

The overall share of land used for crops and pastures is increasing worldwide (Winkler, et al., 2021). However, global cropland per capita is steadily decreasing as the world populations continue to grow (FAO, 2021), leading to a rapid increase in the global use of pesticides and fertilizers (Sharma, et al., 2019). In addition, climate change is increasing the average temperature of most inland water bodies (O'Reilly, et al., 2015). Together, these multiple stressors may severely affect aquatic ecosystems in agricultural areas. Many of these agroecosystems are shallow ponds or slow-flowing streams and are characterized by the alternative dominance of submerged macrophytes, periphyton or phytoplankton, competing for light and nutrients as major resources (Vasconcelos, et al., 2016). Shifts from submerged macrophyte dominance to the less desirable phytoplankton dominance have been reported with increased nutrient loading (Sayer, et al., 2010). Warming is expected to lead to more severe phytoplankton dominance (modeling study by Mooij, et al., 2007) and to increase periphyton growth (Mahdy, et al., 2015; Kazanjian, et al., 2018), resulting in an overall weakening of the resilience of macrophyte-dominated systems to nutrient loading impacts (Meerhoff, et al., 2022). Additional stressors such as pesticides, however, may antagonistically interact with temperature and nutrient loading, modulating the competition for resources between aquatic primary producers (Polst, et al., 2022). Understanding the overall response of these ecosystems to multiple stressors is therefore challenging (Jackson, et al., 2015) but crucial to predict future changes in their functioning (Hilt, et al., 2017) and services (Janssen, et al., 2021).

A first experiment investigating responses of the different primary producers typical for shallow aquatic systems to multiple stressors revealed a direct positive effect of co-occurring nitrate and pesticides on phytoplankton, with no significant effect of warming (Allen, et al., 2021). A subsequent study, involving also primary consumers, showed that pesticides and nitrate may interact synergistically to reduce macrophyte dominance, and that pesticides and warming may have especially strong but opposite effects on specific macrophyte species (Vijayaraj, et al., 2022). Climate warming has even been shown to lower critical thresholds for shifts in dominance between aquatic primary producers (Polst, et al., 2022). These differences may derive from the differential sensitivities to toxicants of different groups and species of primary producers (Giddings, et al., 2013; Fairchild, et al., 1998), some of which may be more tolerant or have the ability to acclimate or adapt to them at different temperature-dependent rates (Larras, et al., 2013; Chalifour & Juneau, 2011). Additional trophic levels, the structure of the ecosystem and the trophic status of the water body may also play a role (Wendt-Rasch, et al., 2004), further complicating the analysis of direct and indirect effects of multiple stressors that may lead to complex responses of shallow aquatic ecosystems.

Reductionist factorial laboratory approaches are useful to address these complex. However, time and budget constraints, and the invasive nature of macrophyte sampling often limit both the number of testable stressor combinations and the frequency of sampling that would be needed to understand community dynamics. Combining experiments with process-based models may be a helpful tool to reveal variable trajectories, to test for critical threshold values, and to disentangle indirect stressor effects. Such models, however, need to be developed based on the specific research questions and the experimental set-up, i.e., custom-tailored to avoid running into equifinality issues (López Moreira M., et al., 2021). The empirical results, in turn, allow for subsequent model refinement, calibration and validation (e.g., Kim, et al., 2021), an iterative process that builds upon every new insight and gained expert knowledge (Jakeman, et al., 2006).

In this study, we combined a microcosm experiment on multiple stressor effects in shallow aquatic ecosystems with simulations we ran with a custom-tailored process-based model. We developed this model to investigate the response of the different primary producer groups (submerged macrophytes, periphyton and phytoplankton) to the combined effects of nitrate and pesticides typically found in agricultural run-off (ARO) with or without effects of climate warming. We hypothesized: 1) that ARO differentially affects primary producer groups increasing the likelihood of phytoplankton dominance in shallow aquatic ecosystems; 2) that warming facilitates this process and 3) that custom-tailored process-based models can support mechanistic understanding of experimental results through scenario comparison. First, we developed a simple process-based mathematical model based on the known effects of herbicides, fertilizers and warming on phytoplankton, periphyton and macrophytes. To assess model predictions, we carried out a microcosm experiment testing the response of three submerged macrophyte species (*Myriophyllum spicatum*, *Potamogeton perfoliatus* and *Elodea nuttallii*) and mixtures of phytoplankton and periphyton typically occurring in European freshwaters. We exposed these systems to a gradient of an experimental ARO cocktail, an artificial mix of organic pesticides (an herbicide, an insecticide and a fungicide), copper-(II) sulfate ($CuSO_4$) and potassium nitrate (KNO_3) for 19 days. The ARO mix was applied in a two-factor factorial design (dose-response set-up) at two different temperatures (ambient: 22°C, warming: 26°C). Experimental results led us to refine the process-based model and test eight scenarios of differential herbicide sensitivities among primary producer groups, temperature dependence of the response, and development of tolerance to the herbicide for three cases of phytoplankton community composition. Comparing in silico simulations with experimental results allowed us to select the best fit and improved mechanistic understanding of differential sensitivities of phytoplankton, periphyton and submerged macrophytes to combined ARO and warming.

2 Materials and Methods

2.1 Process-based model to simulate the microcosms

2.1.1 Governing and supplementary equations

To simulate the combined effects of ARO and warming on the different groups of aquatic primary producers, we developed a process-based mathematical model, which we implemented in MATLAB (R2020a). The model comprises a series of ordinary differential equations (ODEs) similar to those proposed in previous works (Jäger, et al., 2010; Vasconcelos, et al., 2016). These equations describe the following state variables: the live carbon (C) biomass of phytoplankton (as volume concentration, eq. 1), periphyton, hereafter understood as the growth on the vertical surface of the microcosm vases (as areal density, eq. 2), epiphyton, i.e., periphyton growing on macrophyte surfaces (as areal density, eq. 3) and macrophytes (eq. 4). Because exudates and lysates resulting from growth and senescence, respectively, are known to support microbial communities (Kieft, et al., 2021), the model also includes governing equations for the C content of the cellular exudates of all primary producer groups (as volume concentration, eqs. 5-8) and for their dead C biomass (consistent units, eqs. 9-12). These processes were included because the presence of pesticides in ARO and an increased temperature may have important direct effects on decomposers (e.g., heterotrophic bacteria and fungi) and, consequently, on the rates of nutrient recycling within the microcosms (Nielsen, 2006). Additional governing equations describe the areal density of dead phytoplankton cells accumulating in the sediments (eq. 13) and the volume concentration of dissolved inorganic phosphorus (eq. 14). The resulting system of ODEs is:

$$\begin{cases}
\frac{dC_{live,phyto}}{dt} = \left(p_{phyto} - l_{phyto} - \frac{w_{live,phyto}}{H_V}\right) C_{live,phyto} & (1) \\
\frac{dC_{live,peri}}{dt} = (p_{peri} - l_{peri}) C_{live,peri} & (2) \\
\frac{dC_{live,epi}}{dt} = (p_{epi} - l_{epi}) C_{live,epi} & (3) \\
\frac{dC_{live,macro}}{dt} = (p_{macro} - l_{macro}) C_{live,macro} & (4) \\
\frac{dC_{exu,phyto}}{dt} = l_{exu,phyto} C_{live,phyto} - b_{exu,phyto} C_{exu,phyto} & (5) \\
\frac{dC_{exu,peri}}{dt} = l_{exu,peri} C_{live,peri} \frac{A_{peri}}{V} - b_{exu,peri} C_{exu,peri} & (6) \\
\frac{dC_{exu,epi}}{dt} = l_{exu,epi} C_{live,epi} \frac{A_{epi}}{V} - b_{exu,epi} C_{exu,epi} & (7) \\
\frac{dC_{exu,macro}}{dt} = l_{exu,macro} C_{live,macro} \frac{1}{V} - b_{exu,macro} C_{exu,macro} & (8) \\
\frac{dC_{dead,phyto}}{dt} = l_{d,phyto} C_{live,phyto} - \left(\frac{w_{dead,phyto}}{H_V} + b_{dead,phyto}\right) C_{dead,phyto} & (9) \\
\frac{dC_{dead,peri}}{dt} = l_{d,peri} C_{live,peri} - b_{dead,peri} C_{dead,peri} & (10) \\
\frac{dC_{dead,epi}}{dt} = l_{d,epi} C_{live,epi} - b_{dead,epi} C_{dead,epi} & (11) \\
\frac{dC_{dead,macro}}{dt} = l_{d,macro} C_{live,macro} - b_{dead,macro} C_{dead,macro} & (12) \\
\frac{dC_{dead,sed,phyto}}{dt} = w_{live,phyto} C_{live,phyto} + w_{dead,phyto} C_{dead,phyto} - b_{dead,phyto} C_{dead,sed,phyto} & (13) \\
\frac{dP_d}{dt} = S_{phyto} + S_{peri} + S_{epi} + S_{macro} & (14)
\end{cases}$$

where the parameters $p_{i=phyto,peri,epi,macro}$ and $l_{i=phyto,peri,epi,macro}$ (eqs. 1-4) are variable total unit gain and unit loss rates of C biomass of each group; $w_{live,phyto}$ (eqs. 1,13) and $w_{dead,phyto}$ (eqs. 9,13) are constant sinking velocities of live and dead phytoplankton cells, respectively; H_V is the constant height of water in the microcosm of constant water volume V; the $l_{exu,i=phyto,peri,epi,macro}$ (eqs. 5-8) are the variable unit background exudation rates of each group; the $b_{exu,i=phyto,peri,epi,macro}$ (eqs. 5-8) are the variable unit biodegradation rates of exudates; the $b_{dead,i=phyto,peri,epi,macro}$ (eqs. 9-13) are the variable unit biodegradation rates of dead biomass; A_{peri} is the constant area of the microcosm surface where wall periphyton can grow; and A_{epi} the variable area of macrophyte surfaces where epiphyton can grow, formulated as a function of macrophyte biomass. The time coordinate and all state variables (governing eqs. 1-14) are listed and described in Table 1. Primary auxiliary variables, i.e., those appearing in the governing equations of the model, are listed and described in Table 2. The formulation of all secondary auxiliary variables appearing in the equations of primary auxiliary variables is presented as part of the supplementary information (SI), as well as the chosen values of all model parameters (Tables S1 to S6) and the initial values of all state variables (Table S7).

Table 1. Description, units of measurement of the time coordinate and state variables, and reference to the governing equations.

Variable	Description	Units	Formulation
Time coordinate			
t	Time coordinate, with origin at the start of the simulated period and positive direction forward	s	-
State variables			
$C_{live,phyto}$	Volume concentration of live carbon biomass of phytoplankton	$\text{mg C}\cdot\text{m}^{-3}$	Eq. 1
$C_{live,peri}$	Areal density of live carbon biomass of periphyton	$\text{mg C}\cdot\text{m}^{-2}$	Eq. 2
$C_{live,epi}$	Areal density of live carbon biomass of epiphyton	$\text{mg C}\cdot\text{m}^{-2}$	Eq. 3
$C_{live,macro}$	Live carbon biomass of macrophytes	mg C	Eq. 4
$C_{exu,phyto}$	Volume concentration of carbon in exudates of phytoplankton	$\text{mg C}\cdot\text{m}^{-3}$	Eq. 5
$C_{exu,peri}$	Volume concentration of carbon in periphyton exudates	$\text{mg C}\cdot\text{m}^{-3}$	Eq. 6
$C_{exu,epi}$	Volume concentration of carbon in epiphyton exudates	$\text{mg C}\cdot\text{m}^{-3}$	Eq. 7
$C_{exu,macro}$	Volume concentration of carbon in macrophyte exudates	$\text{mg C}\cdot\text{m}^{-3}$	Eq. 8
$C_{dead,phyto}$	Volume concentration of dead carbon biomass of phytoplankton	$\text{mg C}\cdot\text{m}^{-3}$	Eq. 9
$C_{dead,peri}$	Areal density of dead periphyton biomass	$\text{mg C}\cdot\text{m}^{-2}$	Eq. 10
$C_{dead,epi}$	Areal density of dead epiphyton biomass	$\text{mg C}\cdot\text{m}^{-2}$	Eq. 11
$C_{dead,macro}$	Dead macrophyte biomass	mg C	Eq. 12
$C_{dead,sed,phyto}$	Areal density of dead carbon biomass of phytoplankton that is stored in the sediments	$\text{mg C}\cdot\text{m}^{-2}$	Eq. 13
P_d	Volume concentration of dissolved inorganic phosphorus	$\text{mg P}\cdot\text{m}^{-3}$	Eq. 14

Table 2. Description, units of measurement of primary auxiliary variables (appearing in the governing equations), and reference to their formulation.

Variable	Description	Units	Formulation
p_{phyto}	Fractional growth rate of carbon biomass of phytoplankton groups	s^{-1}	Eq. S15
p_{peri}	Fractional growth rate of carbon biomass of periphyton	s^{-1}	Eq. S19
p_{epi}	Fractional growth rate of carbon biomass of epiphyton	s^{-1}	Eq. S20
p_{macro}	Fractional growth rate of carbon biomass of macrophytes	s^{-1}	Eq. S25
$l_{i=phyto,peri,epi,macro}$	Fractional loss rate of carbon biomass of primary producers	s^{-1}	Eq. S28
$l_{d,i=phyto,peri,epi,macro}$	Fractional death rate of carbon biomass of primary producers	s^{-1}	Eq. S29
$l_{exu,i=phyto,peri,epi,macro}$	Fractional exudation rate of carbon biomass of primary producers	s^{-1}	Eq. S30
$b_{dead,i=phyto,peri,epi,macro}$	Fractional biodegradation rate of dead carbon biomass of primary producers	s^{-1}	Eq. S34
$b_{exu,i=phyto,peri,epi,macro}$	Fractional biodegradation rate of cellular exudates of primary producers	s^{-1}	Eq. S35
S_{phyto}	Source/sink of phosphorus, phytoplankton	mg $P \cdot m^{-3}$	Eq. S36
S_{peri}	Source/sink of phosphorus, periphyton	mg $P \cdot m^{-3}$	Eq. S37
S_{epi}	Source/sink of phosphorus, epiphyton	mg $P \cdot m^{-3}$	Eq. S38
S_{macro}	Source/sink of phosphorus, macrophytes	mg $P \cdot m^{-3}$	Eq. S39

Note that in the model, periphyton and epiphyton are treated as separate primary producer groups. While periphyton grows on the vertical glass surface of the microcosm, which is of constant area, epiphyton grows on macrophyte surfaces that change over time as macrophytes develop, and we implemented a space limitation factor in the auxiliary equation for the growth rates of periphyton and epiphyton (eqs. S19 to S22). Additionally, the growth rates of all primary producers are also dependent on nutrient and light availability, and are affected by temperature. On this note, in general, all modelled processes are accelerated under warmer conditions, as described in the SI. The effect of the herbicide was modeled based on log-logistic dose-response curves that are common in toxicokinetic studies (e.g., Copin & Chèvre, 2015). These curves were made specific to each group of primary producers, as described in the SI.

2.2 Microcosm experiment

2.2.1 Microcosm setup

To mimic shallow aquatic ecosystems, we used microcosms based on the OECD Guideline 239 *Water-Sediment Myriophyllum Spicatum Toxicity Test* (Allen, et al., 2021; Vijayaraj, et al., 2022; and Polst, et al., 2022). The microcosms consisted of glass vases (diameter: 25 cm, height: 40 cm, manufacturer: Sandra Rich, Germany) containing a glass bowl (diameter: 14 cm, height: 8 cm, manufacturer: Sandra Rich, Germany) filled with 20% Kaolin (Imerys, France), 5% peat (<1 mm), 1% nettle powder, 74% quartz sand (grain size fraction <0.2 mm, manufacturer: Schicker Mineral GmbH, Germany) and a 2 cm layer of quartz sand (<0.2mm) on top to prevent resuspension of the sediment.

Three submerged macrophyte species that are common in temperate eutrophic freshwater ecosystems (Hilt, et al., 2018) were collected from nearby eutrophic water bodies: *Myriophyllum spicatum* and *Elodea nuttallii* from Lake Müggelsee (Germany) and *Potamogeton perfoliatus* from River Spree (Germany). Two apical shoots of 8 cm (*M. spicatum*, *E. nuttallii*) or 10 cm (*P. perfoliatus*) were planted into the sediments of each microcosm.

All microcosms were filled with 8 L of Volvic® mineral water (Danone Waters Deutschland GmbH, Germany) to ensure homogeneity and low initial nutrient concentrations (7.3 mg $NO_3^{-}\cdot L^{-1}$). After one week of initial adjustment of the system, the following species of photoautotrophic microorganisms were added, that are typical of shallow freshwater ecosystems: four planktonic species

– including two cyanobacteria (*Chroococcus minutus* and *Anabaena* sp. PCC7210) and two green algae (*Scenedesmus obliquus* and *Desmodesmus subspicatus*) – and four benthic species – including a diatom (*Nitzschia palea*), two filamentous green algae (*Uronema* sp. and *Oedogonium* sp.) and a cyanobacterium (*Komvophorum* sp.). The species were selected and grown in Volvic® water prior to inoculation, and then combined for an inoculum with a total biovolume of 1.25×10^9 µm^3. Three plastic strips (30×2.5 cm) were fixed in the sediment bowl and attached to the inner glass vase, providing a surface for periphyton growth (150 cm^2). An aeration system was constructed to ensure mixing of the water within each microcosm using air pumps.

The microcosms were placed inside two climate chambers, each containing a reference temperature sensor in one of the microcosms. Heating and cooling of the microcosms happened via air temperature changes. A 16h:8h light:dark cycle was applied using luminescent light with a mean of 77.2 ± 9.9 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ measured at the water surface.

2.2.2 ARO composition

The artificial mixture of pesticides representing a characteristic agricultural run-off (ARO) was selected as in our previous studies (e.g., Allen, et al., 2021), including three organic pesticides – terbuthylazine (selective chloro-s-triazine herbicide, PSII inhibitor, CAS number 5915-41-3), pirimicarb (fast-acting selective carbamate insecticide, AChE inhibitor, CAS number 23103-98-2) and tebuconazole (triazole fungicide, demethylation inhibitor, CAS number 107534-96-3) – copper (inorganic pesticide, as $CuSO_4$) and nitrate (fertilizer, as KNO_3). The initial concentrations of pesticides were based on dose-response assays and subsequent EC20 calculations (as described in Allen, et al., 2021). An initial concentration of 9 $\text{mg}\cdot\text{L}^{-1}$ NO_3-N was selected as representative for nitrogen concentrations in small lakes in agricultural catchments during spring and summer (Xu, et al., 2014; James, et al., 2005). For the application in the microcosms, the organic pesticides were diluted in dimethyl sulfoxide (DMSO, Sigma-Aldrich, 67-68-5, final concentration in the microcosm 0.1%). $CuSO_4$ and KNO_3 were diluted in ultrapure water.

2.2.3 Two-factor factorial dose-response design

The experiment was conducted in two climate chambers (22°C, 26°C) and in each, we followed a dose-response setup for ARO with one control and five concentration levels (ARO 1, 2, 4, 8, 16; enrichment factor of 2). The ARO mixture and concentrations were chosen based on former work by Allen, et al. (2021). The ARO concentration used in their study was set as intermediate treatment concentration in our experiment (ARO 4) with two higher and two lower concentrations for the gradient. Each treatment had five to seven replicates.

2.2.4 Time schedule

The bowls filled with sediment were prepared and pre-wetted with Volvic® mineral water and stored in dark conditions at 22°C for two days before macrophytes were planted and microcosms were filled with Volvic® mineral water. Afterwards, macrophytes were given two weeks to adjust to the conditions. The inoculum of microscopic primary producers and plastic strips were added one week before the start of the experiment. At the start of the experiment, treatments were applied by adding the ARO mix to the microcosms and increasing the temperature in one of the climate chambers to 26°C. Hereafter, nutrients in the form of a KNO_3 and KH_2PO_4 mixture as in Allen et al. (2021) were added twice a week, simulating repeated nutrient loading to compensate for a fast nutrient uptake by wall periphyton, and thus ensure sufficient nutrient availability to sustain further primary producer growth. On day 16, 17 and 18, we sampled periphyton and phytoplankton, and took water samples for chemical analysis (day 18). From day 19 to day 23, the lights were turned off and sampling of the macrophytes took place (Figure S1).

2.2.5 Sampling methods

Biomass sampling of primary producers

Periphyton was brushed off from the plastic strips using a toothbrush and suspended in Volvic® water. This periphyton-suspension then was filtered with pre-weighted glass fiber filters (0.7 µm), dried at 60°C and weighted to determine the periphyton dry weight. To determine phytoplankton biomass, water samples were filtered using pre-weighted glass fiber filters (0.7 µm), dried at 60°C and weighted. Macrophytes were removed from their microcosm for the final sampling and the aboveground part of each macrophyte specimen was separately packed in paper bags, dried at 60°C for two days and then weighted to determine the dry weight of each stem.

Water sampling for pesticide concentrations

Two hours and 18 days after application of the treatments, 4 mL-water subsamples of three microcosms per ARO treatment were taken and frozen at −20°C. Later, the samples were filtered (0.2 µm) and analyzed for their concentration of the three pesticides used in the ARO mixture. Measurement of the pesticides was conducted using a UltiMate3000 HPLC System combined with an LTQ-OrbiTrap XL (Thermo Scientific, USA). These samples were then analyzed with an UltiMate3000 HPLC System (column: Phenomenex, Art.-No. 00B-4462-Y0) and an attached LTQ Orbitrap XL (Thermo Scientific) operated in positive ionization mode.

2.2.6 Statistical analyses

Non-parametric statistical tests were used to test for differences between treatments related to the ARO application or the temperature. These analyses were conducted using the software R (R Core Team, 2020; v4.0.0). For each of the three primary producer groups differences in biomass were tested via the Kruskal-Wallis test followed by the Dunn-post-hoc test without further correction due to the low number of treatments.

Further, biomass of each primary producer group was extrapolated to the whole biomass per microcosm, including periphyton growing on the inner walls of the microcosm. Based on this total microcosm primary producer biomass, the effect sizes (as Hedges' *g*) were calculated using the *esc* package (Lüdecke, 2019; v0.5.1). Using the mean biomass of each primary producer group as well as the total biomass of all primary producer groups together, pie charts were created for simplified presentation of the share of each primary producer group within each treatment.

2.3 Model refinement and assessment against experimental results

The information we progressively gained over the course of the experiment and after experimental results became available allowed for further refinement of the model to account for some processes like the decay of toxicant concentrations over time (revealed by water sampling results), or to better represent processes like nutrient uptake by wall periphyton, nutrient recycling due to biodegradation, and trait-based phytoplankton community adaptation by splitting the phytoplankton compartment into two groups. Group α was conceived as a fast-growing phytoplankton group that was highly sensitive to the herbicide (i.e., low EC50 value), whereas group β was made up of slow-growing phytoplankton that were resistant to the herbicide (i.e., a much higher EC50 value than group α).

Moreover, the log-logistic dose-response curves were made variable in time to account for the potential development of tolerance to the herbicide and/or acclimation of the organisms to better cope with the combined stressors. We assumed these adaptation and acclimation processes to be directly linked to the generation time of the organisms, i.e., a faster for microscopic primary producers. This was achieved by implementing dynamic parameters for the dose-response curves, as described in the SI. The potential of the refined model to simulate community adaptation, tolerance development and organism acclimation led us to design and test the model under different scenarios of herbicide sensitivity, temperature dependence of primary producer response and community adaptation.

2.3.1. Scenarios of differential sensitivities, temperature dependence and community adaptation

We designed eight different scenarios to test our hypotheses and to mechanistically understand experimental results. These scenarios related to: a) whether microscopic primary producers (phytoplankton group α, periphyton and epiphyton) were equally, more or less sensitive to the herbicide than macrophytes; b) whether these sensitivities were influenced by temperature or not; and c) whether sensitivities decreased over time or not as a result of the development of tolerance to the herbicide by acclimation of microscopic primary producers to environmental conditions or succession towards more tolerant species (Table 3).

In scenarios A1 and A2, all primary producer groups were equally sensitive to the herbicide except for phytoplankton group β (conceived as slow-growing and resistant to the herbicide). In scenarios B1 and B2, macrophytes were more sensitive to the herbicide than all other primary producer groups. In scenarios C1 and C2, microscopic primary producers were more sensitive to the herbicide than macrophytes, with the exception of phytoplankton group β, which, again, was more resistant than all other groups.

In all aforementioned scenarios (A1-2, B1-2 and C1-2), we assumed that no development of tolerance to the herbicide by acclimation or adaptation to environmental conditions (Lips, et al., 2022) occurred over the course of the experiment, i.e., sensitivities do not decrease over time. In scenarios D1 and D2, however, we account for this by implementing decreasing herbicide sensitivities over the course of the exposure for all microscopic primary producers. In these two scenarios, phytoplankton group α, periphyton and epiphyton start off being more sensitive to the herbicide than macrophytes, but end up becoming less sensitive than macrophytes by the end of the simulated period as a result of tolerance development. Here too, phytoplankton group β starts off being less sensitive than all other groups, and becomes increasingly tolerant over time.

Table 3. Scenarios of differential sensitivities, temperature dependence, community adaptation and organism acclimation.

Scenario	Herbicide sensitivity by group	Effect of temperature on herbicide sensitivity
A1	Fast-growing phytoplankton (group α), wall periphyton, epiphyton and macrophytes are equally sensitive to the herbicide. Slow-growing phytoplankton (group β) are less sensitive to the herbicide than all other groups. Sensitivities do not decrease over time for any group (no community adaptation or acclimation to the herbicide over time).	NO
A2		Sensitivities are higher at the lower temperature (22°C)
B1	Macrophytes are more sensitive to the herbicide than all microscopic primary producers. Slow-growing phytoplankton (group β) are less sensitive to the herbicide than all other groups. Sensitivities do not decrease over time (no community adaptation or acclimation to the herbicide).	NO
B2		Sensitivities are higher at the lower temperature (22°C)
C1	Fast-growing phytoplankton (group α), wall periphyton and epiphyton are more sensitive to the herbicide than macrophytes. Slow-growing phytoplankton (group β) are less sensitive to the herbicide than all other groups. Sensitivities do not decrease over time (no community adaptation or acclimation to the herbicide).	NO
C2		Sensitivities are higher at the lower temperature (22°C)
D1	Initially, fast-growing phytoplankton (group α), periphyton and epiphyton are more sensitive to the herbicide than macrophytes. These groups become less sensitive to the herbicide over time to become less sensitive than macrophytes by the end of the exposure. From the beginning of the exposure, low-growing phytoplankton (group β) are less sensitive to the herbicide than all other groups and become increasingly tolerant to it over time.	NO
D2		Sensitivities are higher at the lower temperature (22°C)

In all scenarios indexed with "1", we assumed that temperature had no effect at all on herbicide sensitivities, whereas in all scenarios indexed with "2", sensitivities to the herbicide were assumed to be higher at the lower temperature (22°C) than at the higher temperature (26°C), following empirical evidence by Tasmin et al. 2013. Moreover, this is consistent with the fact that metabolic processes are in general accelerated at higher temperatures (Pedrosa Gomes & Juneau, 2017; Clarke & Fraser, 2004).

To further account for possible community adaptation and succession of phytoplankton to the applied stressors related to an increasing dominance of the more tolerant species (Lips, et al., 2022; Tlili, et al., 2016; and Blanck, 2002), we ran three sets of simulations under all eight scenarios. Set 1: a single phytoplankton group that is fast-growing and highly sensitive to the herbicide, hereafter referred to as group α; Set 2: a single phytoplankton group that is slow-growing but much less sensitive to the herbicide, hereafter referred to as group β; Set 3: a mixed community of both phytoplankton groups α and β, as previously described.

2.3.2. Assessment of model simulation results

We assessed the goodness of fit between model results and the observations from the subsequent microcosm experiment following a pattern-oriented model validation approach that took the ARO treatment as predictor of final biomass values for each of the following three primary producer groups: phytoplankton, periphyton (wall growth in the model, periphyton strip growth in the experiment) and macrophytes. The resulting patterns were compared qualitatively in terms of the similarity between simulated and observed response curves and quantitatively on the basis of the correlation coefficients calculated for all scenarios between model results and observations.

3 Results

3.1 Microcosm experiment

3.1.1 Biomass of different primary producer groups

At 22°C, all ARO treatments showed significantly lower macrophyte biomass than the controls ($p < 0.05$), but did not significantly decrease between the five ARO treatments. At 26°C, macrophyte biomass across all ARO concentrations was not significantly different from controls and the corresponding ARO concentrations at 22°C (Figures 1 and 6, and Table 5). At 22°C, phytoplankton biomass showed a significant increase compared with the controls at the second tested ARO concentration level (ARO 2) and higher (apart from ARO 4). At 26°C, phytoplankton biomass was significantly higher at ARO 4 and ARO 16. No significant differences between the corresponding ARO concentrations of both temperatures were detected (Figures 1 and 6, Table 5). Periphyton biomass was not significantly affected by ARO at 22°C and 26°C, likely due to the high variation between replicates in the controls. Effect sizes were still lower than −1 at the highest tested ARO level for both temperatures (Figures 1 and 6, and Table 5).

The average accumulated microcosm biomass for all primary producers decreased in the ARO treatments compared with the control at 22°C. At 26°C the accumulated microcosm biomass of the ARO treatments was higher than their control at 26°C and the respective ARO treatments at 22°C. (Figure 2). In the control treatment, macrophytes dominated with periphyton having the second highest share. The share of both, macrophytes and periphyton decreased with ARO treatments due to increasing phytoplankton abundance, with the lowest share of macrophytes on the accumulated microcosm biomass at ARO 4 (Figure 2). The share of phytoplankton increased at 26°C.

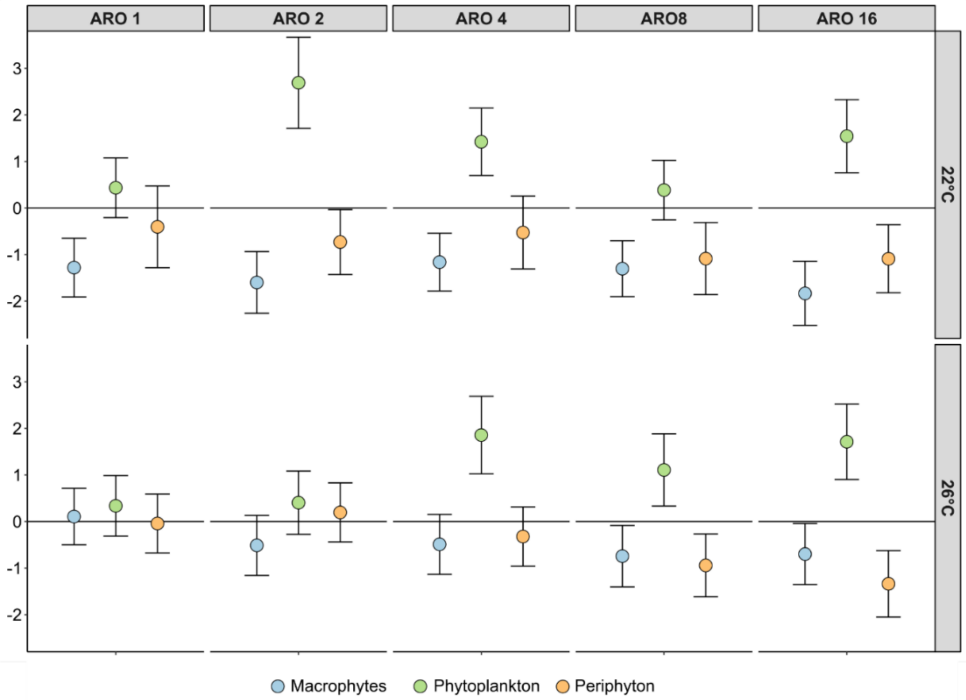

Figure 1. Effect sizes (Hedges' *g*) and its standard deviation for the response of different primary producer groups (blue = macrophytes, green = phytoplankton, orange = periphyton) and ARO treatment levels at 22°C (top row) and 26°C (bottom row).

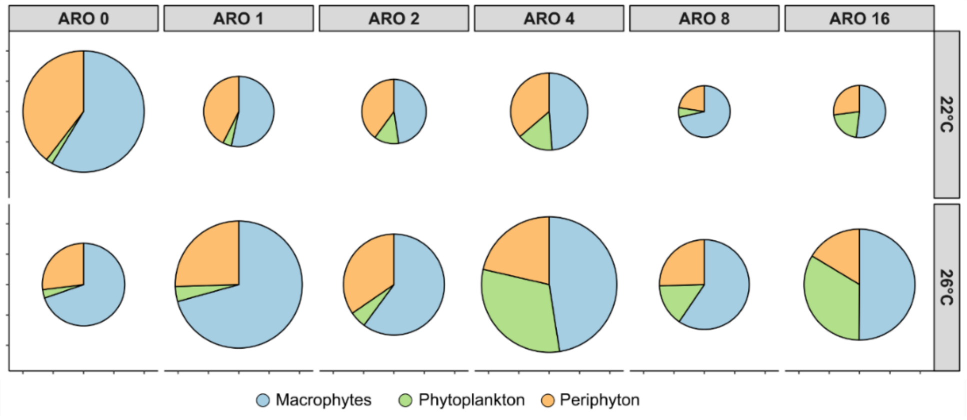

Figure 2: Biomass and proportion of the three primary producer groups upscaled to whole microcosm biomass. Size of the pies and numbers below indicates relative total biomass in comparison with the ARO 0 treatment at 22°C.

3.1.2 Change in pesticide concentrations

After two hours of exposure, the pesticide concentrations were approximately 10% lower than the nominal concentration (Table 4). One microcosm showed higher concentrations than planned (and wrong ratios between the three pesticides) and was, thereafter, excluded from further analysis. At the end of the experiment (after 19 days), between 55-90% of the initial pesticide concentrations were still found. No temperature-related difference in the pesticide concentrations at the end of the experiment was found.

Table 4. Nominal and measured ARO concentrations 2 hours after its application (n=3, ARO 8: n=1, < d.l. = below detection limit). Concentrations in µg L^{-1}.

Treatment	Terbuthylazine	Pirimicarb	Tebuconazole	Copper	NO_3-N
Control	0 (< d.l.)	0 (< d.l.)	0 (< d.l.)	0 (< d.l.)	0 (< d.l.)
ARO 1	0.75|0.64±0.01	3.75|3.82±0.22	22.5|21.11±1.21	10.5	2250
ARO 2	1.5|1.28±0.06	7.5|7.24±0.14	45|38.07±4.00	21	4500
ARO 4	3|2.74±0.06	15|15.56±0.62	90|83.46±6.56	42	9000
ARO 8	6|5.70±0.00	30|31.07±0.00	180|171.80±0.00	84	18000
ARO 16	12|11.6±+0.59	60|63.64±3.02	360|323.03±23.06	168	36000

3.2 Process-based microcosm model

Our custom-tailored process-based model showed that, in all simulation sets, periphyton and macrophyte carbon biomass values per unit volume (mg C·m^{-3}) decreased along the axis of increasing ARO concentrations at both 22°C and 26°C. Whether this decrease was more or less pronounced, and the pattern itself of the response ultimately depended on how well phytoplankton developed, with a stronger decrease in benthic primary producers when phytoplankton performed better (Figures 3, 4 and 5). For simulation set 1, a community of only fast-growing phytoplankton that are highly sensitive to the herbicide, none of the eight scenarios resulted in phytoplankton response patterns similar to those observed empirically, i.e., from the microcosm experiment (Figures 1 and 6). Empirical and simulated phytoplankton response patterns to ARO were more comparable for simulation sets 2 (group β only) and 3 (mixed community of groups α and β), but only under warm conditions (Figures 5 and 6). Correlation coefficients calculated for the 3 sets also showed that the best fits across all primary producer groups were obtained for sets 2 and 3 when herbicide sensitivities were assumed to be temperature dependent and to decrease over time as a result of tolerance development and/or acclimation to the environment, i.e., scenario D2 (Table 5).

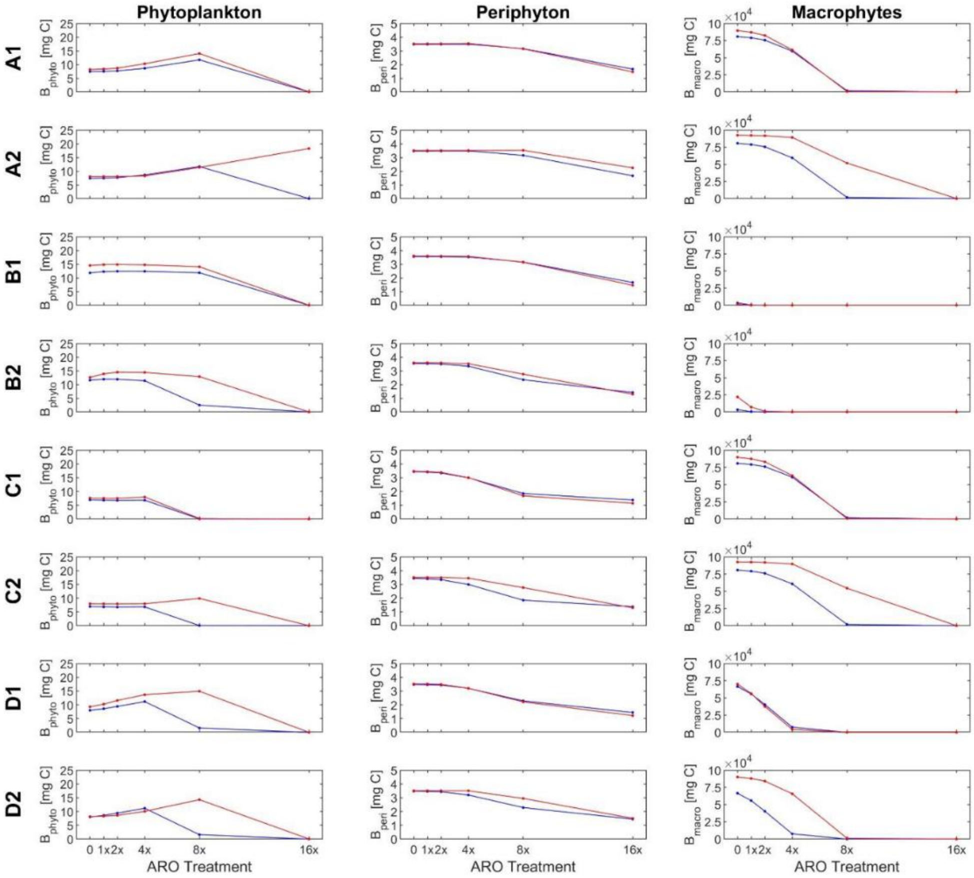

Figure 3. Response of primary producer groups under the eight scenarios for the case of a single, fast-growing phytoplankton group that is highly sensitive to the herbicide (simulation set 1, group α only) under ambient (22°C, blue) and warm (26°C, red) temperature conditions.

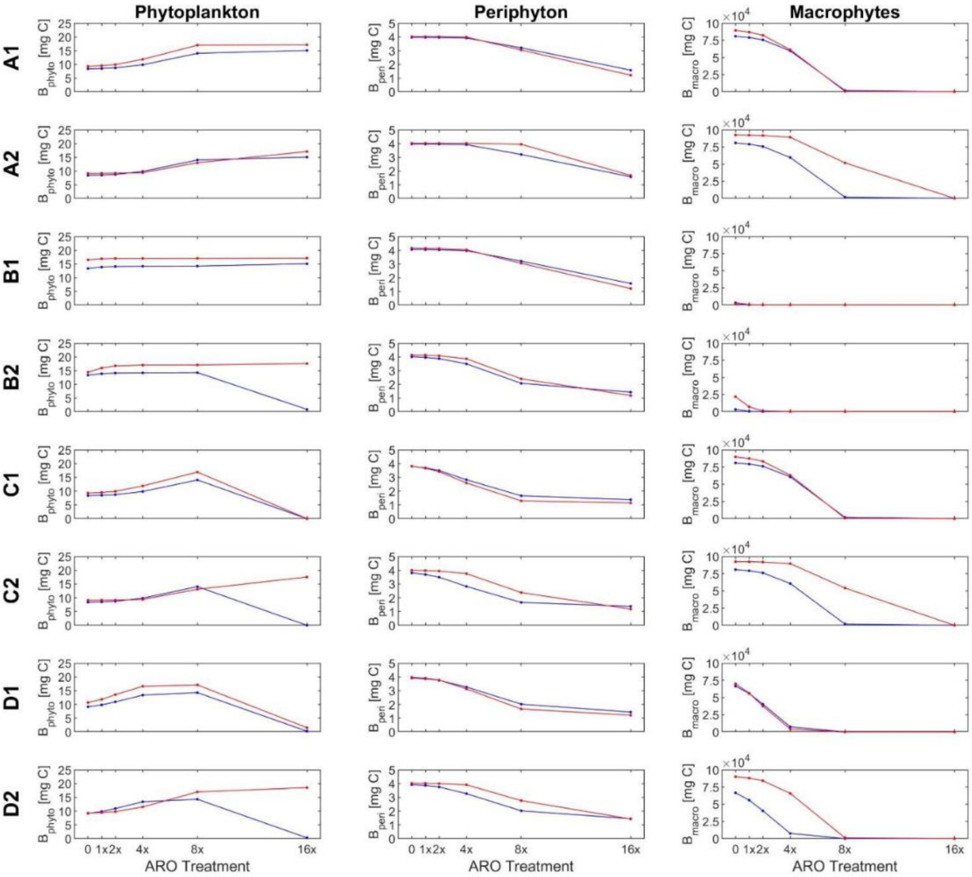

Figure 4. Response of primary producer groups under the eight scenarios for the case of a single, slow-growing phytoplankton group that is highly resistant to the herbicide (simulation set 2, group β only) under ambient (22°C, blue) and warm (26°C, red) temperature conditions.

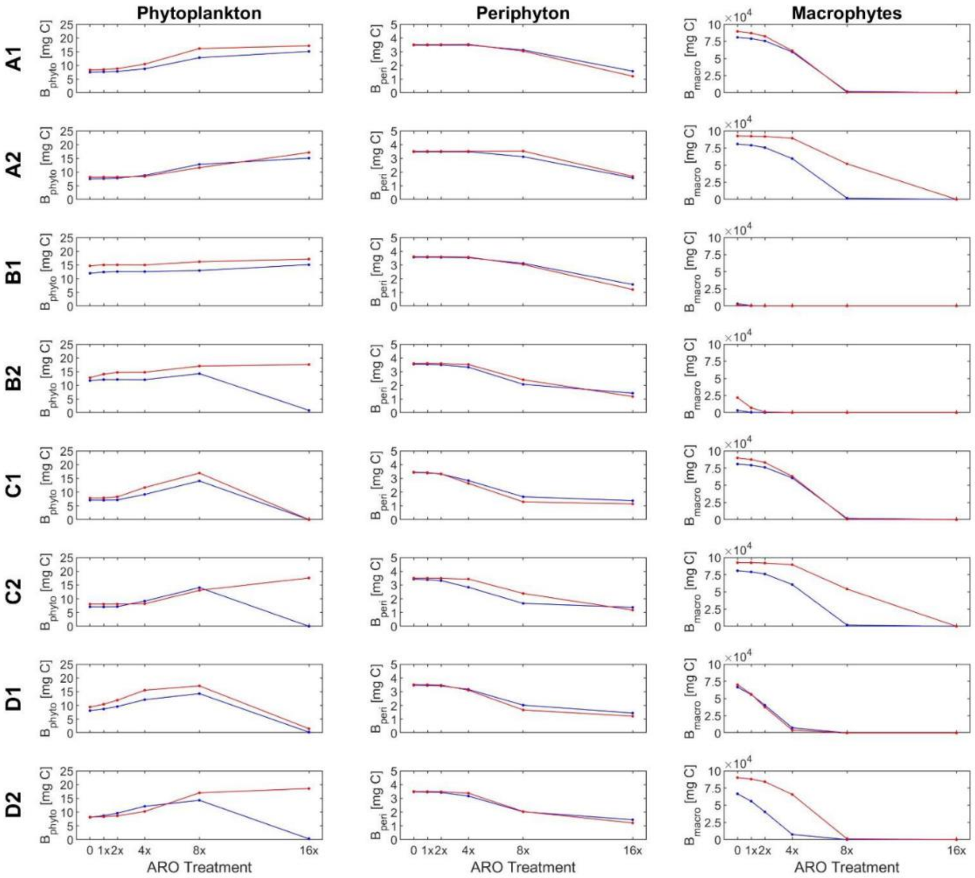

Figure 5. Response of primary producer groups under the eight scenarios for the case of a mixed phytoplankton community (simulation set 3, groups α and β) under ambient (22°C, blue) and warm (26°C, red) temperature conditions.

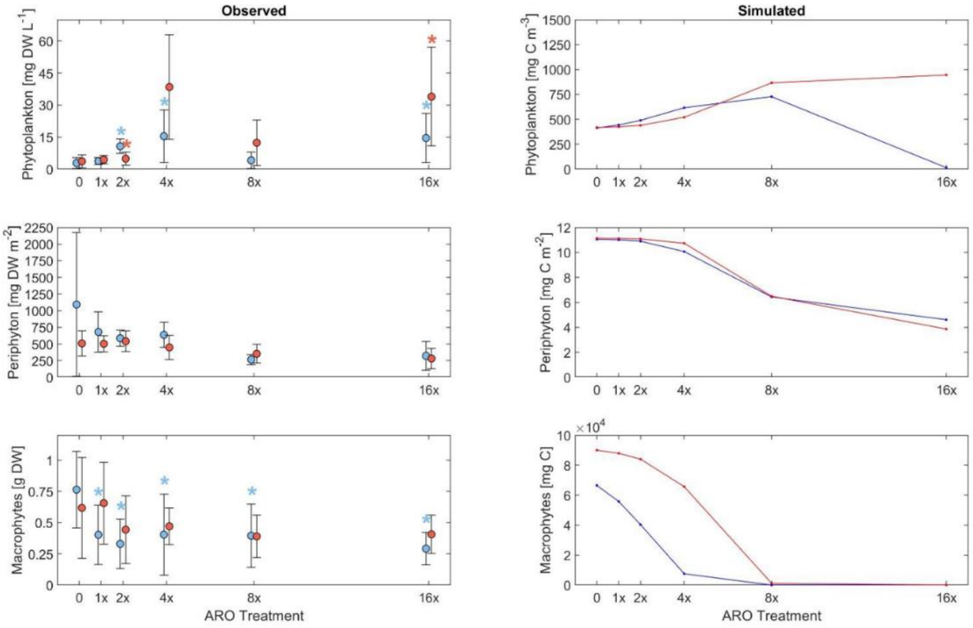

Figure 6. Response of different primary producer groups to multiple stress by increasing concentrations of pesticides and nutrients in agricultural runoff (ARO) and warming (+4°C) observed in experimental microcosms (left) and simulated (right) under the scenario of best fit (model scenario D2) for warm (26°C, red) and ambient (22°C, blue) temperature treatments.

Table 5. Correlation coefficients between simulated and observed patterns under eight scenarios and three cases of phytoplankton community composition.

Scenario	Phytoplankton		Periphyton		Macrophytes	
	Cool	Warm	Cool	Warm	Cool	Warm
Set 1: Phytoplankton group *α* only						
A1	-0.501	-0.353	0.561	0.852**	0.470	0.774*
A2	-0.501	0.532	0.561	0.754*	0.470	0.609
B1	-0.479	-0.545	0.584	0.870**	**0.969****	0.579
B2	-0.200	-0.495	0.747*	**0.924****	**0.969****	0.757*
C1	-0.116	-0.308	0.810*	**0.980****	0.467	0.770*
C2	-0.116	-0.534	0.810*	0.914**	0.467	0.599
D1	0.210	-0.301	0.758*	**0.974****	0.831**	0.876**
D2	0.210	-0.362	0.758*	0.893**	0.831**	0.763*
Set 2: Phytoplankton group *β* only						
A1	0.293	0.531	0.640	0.916**	0.470	0.774*
A2	0.293	0.505	0.640	0.779*	0.470	0.609
B1	0.694	0.556	0.655	**0.927****	**0.969****	0.579
B2	-0.484	0.647	0.807*	**0.969****	**0.969****	0.756*
C1	-0.498	-0.338	0.851**	**0.953****	0.467	0.769*
C2	-0.498	0.509	0.851**	**0.967****	0.467	0.599
D1	-0.325	-0.229	0.817**	**0.977****	0.831**	0.876**
D2	-0.325	0.539	0.817**	**0.951****	0.831**	0.764*
Set 3: Mixed community of phytoplankton groups *α* and *β*						
A1	0.330	0.519	0.569	0.863**	0.470	0.774*
A2	0.330	0.524	0.569	0.756*	0.470	0.609
B1	0.553	0.477	0.590	0.879**	**0.969****	0.579
B2	-0.546	0.522	0.781*	**0.951****	**0.969****	0.757*
C1	-0.457	-0.216	0.826**	**0.969****	0.467	0.770*
C2	-0.457	0.479	0.826**	**0.947****	0.467	0.600
D1	-0.342	-0.170	0.850**	**0.978****	0.831**	0.876**
D2	-0.342	0.503	0.850**	**0.966****	0.831**	0.764*

* Significant at a 90% confidence level (*p-value* < .1)

** Significant at a 95% confidence level (*p-value* < .05)

**** (in bold)** Significant at a 99% confidence level (*p-value* < .01)

4 Discussion

To better understand the complex nature of primary producer interactions and responses to multiple stressors we combined indoor microcosm experiments with a custom-tailored process-based model. We confirmed hypotheses 1) that ARO differentially affects primary producer groups increasing the likelihood of phytoplankton dominance in shallow aquatic ecosystems and 3) that custom-tailored process-based models can support mechanistic understanding of experimental results through scenario comparison. Hypothesis 2, a facilitation of shifts to phytoplankton dominance by warming, was only partially confirmed. Here we discuss the respective implications of our findings.

4.1 Agricultural run-off differentially affects primary producers and their interactions

Our results demonstrate that ARO has a different effect on the respective primary producer groups. While we did not observe a full dominance of either primary producer group at the end of the microcosm experiment, an increasing proportion of phytoplankton and decreasing proportion of macrophytes in the total microcosm biomass clearly indicates an increasing risk for the system to shift to full phytoplankton dominance. The overall non-linear increase of phytoplankton biomass with increasing ARO concentrations was most likely caused by nitrate (Scheffer, et al., 1993; Olsen, et al., 2015). Similar microcosm studies testing the same ARO mixture also found an increase in phytoplankton biomass with accompanied decrease in macrophyte biomass when nitrate and pesticides co-occurred (Vijayaraj, et al., 2022; and Polst, et al., 2022). The increase in phytoplankton biomass may have contributed to the observed decline in macrophyte biomass by shading. Regime shifts with macrophyte loss due to nutrient loading are usually caused by increased shading by periphyton and phytoplankton (Phillips, et al., 2016). Yet, at 22°C, a decline in macrophyte biomass was already observed at the lowest ARO concentration, which did not increase phytoplankton biomass, indicating a higher sensitivity of the tested submerged macrophyte species to ARO compared with phytoplankton.

This contrasts the findings of Giddings et al. (2013) who reported a higher sensitivity of phytoplankton species compared with macrophytes for 4 out of 5 photosystem-II-inhibiting herbicides in a meta-analysis based on standardized single-species tests. In longer lasting experiments allowing for multiple generation cycles, microalgae community composition can change via acclimation, adaptation and selection of more tolerant species leading to a pollution-induced community tolerance (Lips, et al., 2022; Lorente, et al., 2015; and Blanck, 2002). Such processes could explain the higher sensitivity and the lower tolerance of macrophytes to ARO in comparison to phytoplankton and periphyton in our experiment. Our results thus suggest that differential sensitivities of primary producers to pesticides can affect their competition for light and facilitate the loss of macrophytes, eventually leading to regime shifts of shallow ecosystems.

4.2 Warming affects the risk of an ARO-induced regime shift to phytoplankton dominance

In our experiment, ARO at both tested water temperatures clearly changed the contributions of different primary producers to the total biomass towards a higher proportion of phytoplankton indicating the risk of an ARO-induced shift to full phytoplankton dominance. However, absolute macrophyte biomass did not respond to ARO in the warmed (+4°C) treatments similar to findings by Allen et al. (2021). Potentially, higher temperatures led to an enhanced growth partially compensating negative pesticide effects on macrophytes. Higher growth rates of macrophytes at higher temperatures are known from other studies (e.g., Zhang, et al., 2022; Zhang, et al., 2019). In contrast, Vijayaraj et al. (2022) and Polst et al. (2022) described a stronger decrease in macrophyte biomass and thus lower threshold levels for ARO-induced shifts to phytoplankton dominance in +4°C treatments. These results may be caused by a lower sensitivity of phytoplankton towards herbicides at elevated temperatures (Tasmin, et al., 2013; Chalifour & Juneau, 2011). Periphyton also seems less sensitive to herbicides at elevated temperatures (Larras, et al., 2013), but we could not confirm this in our nor in related experiments using the same ARO mixture (Polst, et al., 2022; Allen, et al., 2021). Another recent study also found no effect of combined warming, nutrients and pesticides on periphyton and suggested that treatment effects compensated each other (Zhang, et al., 2022). We conclude that warming facilitates macrophyte and phytoplankton growth, which can modulate macrophyte response thresholds to ARO. However, the increasing proportion of phytoplankton in the primary producer community under combined warming and ARO exposure indicates an overall increased risk of macrophyte loss and supported by findings in Vijayaraj et al. (2022) and Polst et al. (2022).

4.3 Process-based models support mechanistic understanding of experimental results

Custom-tailored models of appropriate complexity level, i.e., including just the necessary processes to reproduce empirical observations, are helpful to shed light on the critical mechanisms behind ecosystem-wide responses (López Moreira M., et al., 2021; Vasconcelos, et al., 2016). This is particularly true for addressing the effects of multiple stressors, for which a near infinite number of combinations precludes an exclusive limitation to experiments and for which the knowledge base is just starting to develop. Readily available models would need substantial modifications, recalibration and revalidation before use and may still remain unnecessarily complex for exploratory research. We thus suggest to calibrate and validate newly developed, custom-made simple models with experimental data and run scenarios which allow subsequent model refinements and mechanistic interpretation of model results.

Our pattern-oriented modeling effort allowed for the reconstruction of primary producer temporal dynamics at different levels of approximation to the empirical observations. It also made it possible to assess the trajectories of all state variables (exemplified Figures S3a and S3b) and the most likely limiting conditions to primary producer growth and their change over time (exemplified for epiphyton in Figure S4). Because the empirical data was mostly limited to end-point values, however, model calibration and scenario selection was limited to final biomass results. A series of goodness-of-fit and model efficiency metrics allowed for the quantitative identification and selection of the most plausible scenario that best fitted empirical observations.

Assuming a community of only fast-growing phytoplankton that are highly sensitive to the herbicide (group α), the model was clearly not able to reproduce the pattern arising from the experiment where final biomass values were generally higher for the highest ARO concentrations (Figure 3 and 6). This complex response was better captured under the assumption of a single, slow-growing but relatively resistant phytoplankton group (group β), but only under warm conditions (26°C) (Figure 4). The best overall results, however, were achieved under scenario D2 for a mixed phytoplankton community (groups α and β) (Figure 5).

Under scenario D2, for simulation sets 2 (group β only) and 3 (mixed community of groups α and β), simulated and observed responses were highly positively correlated for both periphyton (lowest correlation coefficient was 0.758 for both sets) and macrophytes (lowest correlation coefficients were 0.817 and 0.850 for sets 2 and 3, respectively). All these correlations were statistically very significant at a confidence level of 90%, 95% or even 99% (Table 5). Phytoplankton responses, however, were only weakly positively correlated in the warming treatment (26°C) and even negatively correlated under ambient temperature (22°C), but none of these correlations were statistically significant. Nevertheless, these values improved marginally for the case of a mixed community (set 3).

In scenario D2, sensitivities to the herbicide of all primary producer groups were dependent on temperature (organisms were less sensitive at the higher temperature). Additionally, herbicide sensitivities of all microscopic primary producers also decreased over time as they became more tolerant to the herbicide. This resulted in a simulated pattern under scenario D2 that was positively correlated with the observed phytoplankton response for the warm temperature treatment. Under scenario D1, where sensitivities were not affected by temperature, a positive correlation could not be achieved for any of the sets.

In our study, the most plausible scenario was the one involving stress-induced phytoplankton tolerance, including organism acclimation and adaptation to ARO as well as stress-induced succession in communities. The relevance of these processes has been shown under the concept of pollution-induced community tolerance (Lips, et al., 2022; Schmitt-Jansen, et al., 2016; and Blanck, 2002). Another recent study reported observed changes in phytoplankton communities of small lentic waters following exposure to agricultural run-off (Wijewardene, et al., 2021) but did not test for changes in tolerance to the pesticides. While designed for a specific experimental set-up, our model can be used for future hypothesis testing in experimental studies with a similar complexity (focused primary producer interactions), but can also be extended to include higher trophic levels.

4.4 Final remarks

Our microcosm experiment revealed a differential response of aquatic primary producers to the combined effects of warming and ARO, potentially leading to phytoplankton dominance, a less desired ecosystem state. At both tested temperatures, phytoplankton was favored and increased its biomass and proportion in the primary producer community with increasing ARO concentrations due to the higher availability of nitrate and ability to adapt to pesticides. Macrophytes became less sensitive to ARO with warming, but may still be weakened due to the increasing proportion of phytoplankton eventually shading out submerged macrophytes. The development of a simple, process-based model allowed understanding the role of community adaptation to ARO, because a scenario including organism acclimation and community adaptation and succession ultimately led to the best fit between predicted and observed responses. Our results highlight the importance of considering stress-induced tolerance development, adaptation and succession when predicting mid- and long-term effects of toxicants on complex primary producer communities that include multiple species. Trait diversity may compensate for the direct negative effects of pesticides on individual species to keep ecosystems productive at a macroscopic scale, especially at higher temperatures and under nutrient-enriched conditions, where ARO may unbalance the system through indirect effects on vulnerable communities like macrophytes.

Author contributions

The experimental part of the study was mostly done by BHP. SH and other colleagues at the IGB Berlin offered help during the final sampling activities of the experiment. The process-based model was developed and implemented by GALMM. SH, FH, MSJ and BHP all provided guidance for the development of the model. GALMM and BHP contributed equally to the final manuscript. EMG contributed to the discussion and editing of the manuscript as Principal Investigator of the CLIMSHIFT project, which funded this work.

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

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Supplemental Material is included at the end of this chapter starting at page 31 of the following manuscript.

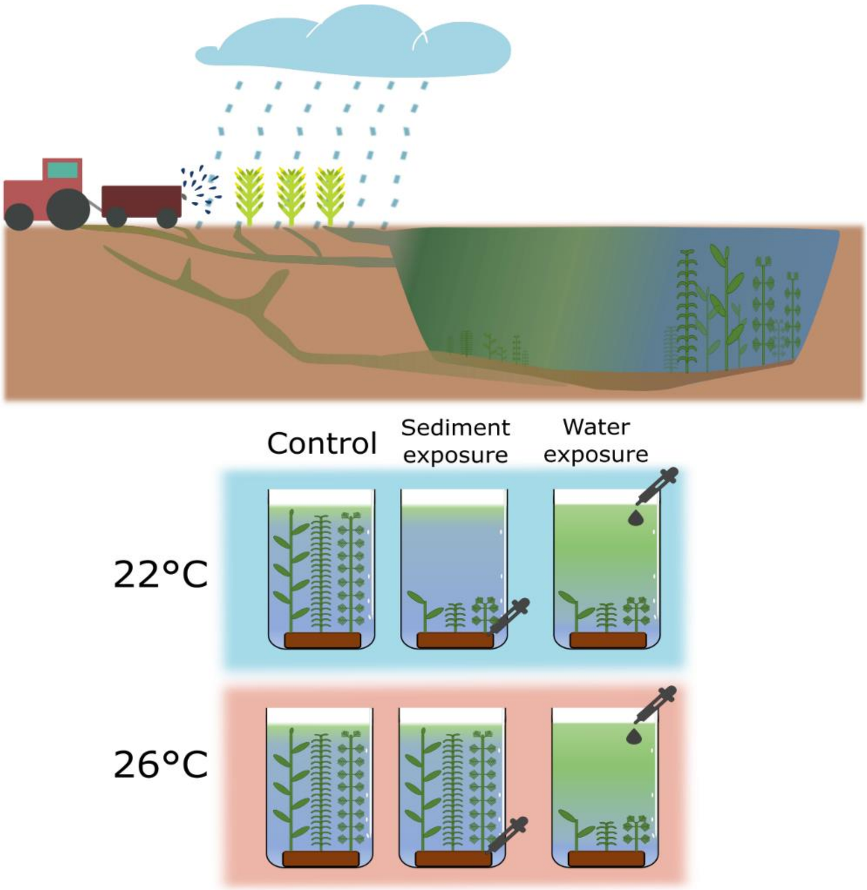

Figure 16 | Graphical abstract for the following *Exposure Pathways* chapter 5.

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

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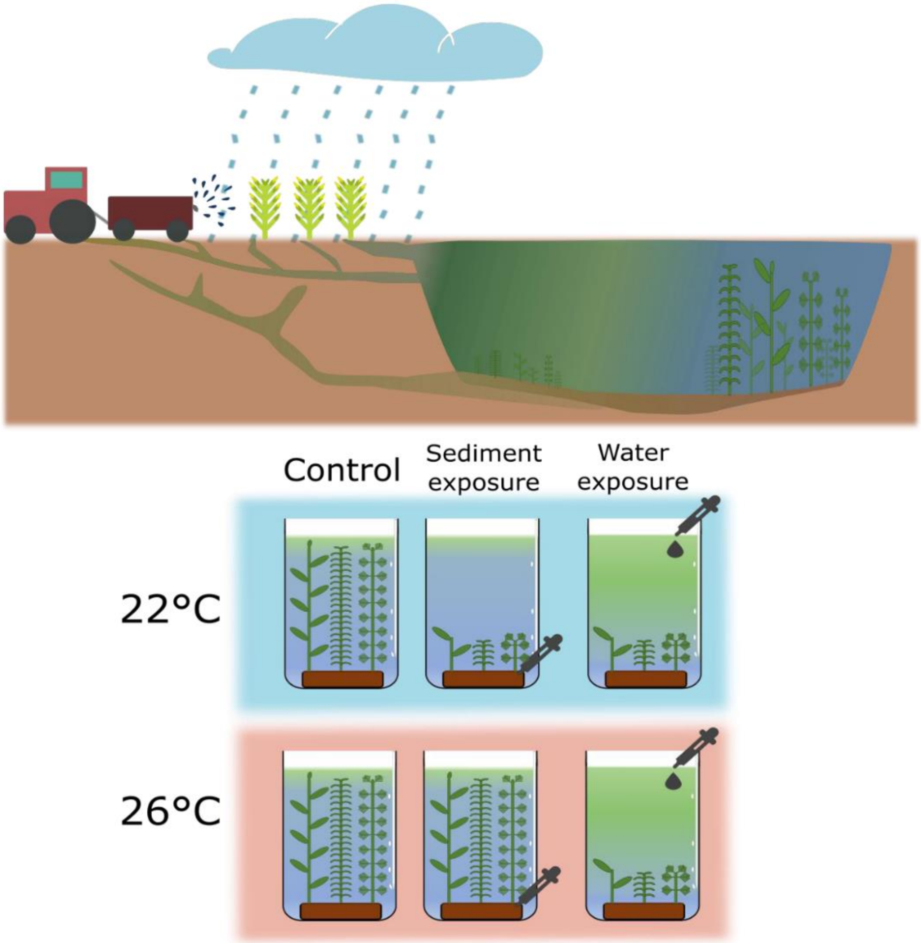
Abstract:

1. Small shallow ponds and lakes are frequent 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 hypothesized 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 regime shifts and alternative stable states of macrophyte- or phytoplankton dominance. We further hypothesized that warming increases nutrient release from sediments and thereby leads to regime shifts.
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 four 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 treatments compared to controls without ARO, indicating a prevalence of negative effects by herbicides and competition for light with other phyto-communities. 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 was not affected by ARO. Higher temperature mitigated ARO-related effects on macrophytes under sediment exposure.
4. *Synthesis and application*. Consequently, ARO poses a strong risk of submerged macrophyte loss and shifts to turbid conditions with phytoplankton dominance in adjacent aquatic ecosystems. Such regime shifts are most likely when primary producers are exposed to ARO dissolved in water, while warming might mitigate these effects. In conclusion, exposure pathways as well as indirect and interacting effects of multiple stressors need to be considered when designing monitoring and appropriate mitigation measures to protect these vulnerably, but frequent aquatic systems in agricultural landscapes.

Keywords: Agricultural run-off, global warming, exposure pathways, shallow lake, regime shift, macrophyte, microalgae

Graphical Abstract:



1. Introduction

Multiple stressors threaten freshwater ecosystems worldwide. For instance, in agricultural landscapes pollution by nutrients and pesticides often co-occur (Birk et al., 2020; Schinegger et al., 2012). Surface as well as subsurface run-off transports these mixtures from agricultural fields into nearby aquatic ecosystems. Often, these affected systems are small, shallow lakes or ponds. However, even though ponds are among the most biodiverse and ecologically important freshwater habitats globally, they are underrepresented in research and monitoring (Hill et al., 2021; Verpoorter et al., 2014). The good ecological state of these systems is characterised by a dominance of macrophytes but threatened by a regime shift to phytoplankton dominance due to eutrophication with significant consequences for ecosystem functioning (Hilt et al., 2017; Scheffer et al., 1993). However, also co-exposure to pesticides via the water phase promotes regime shifts from macrophyte to phytoplankton dominance in small, shallow water bodies (Polst et al., 2022; Vijayaraj et al., 2022).

Nutrients and pesticides transported by agricultural run-off (ARO) could end up directly in the water column (Ulrich et al., 2013), or be rapidly transferred to sediments either absorbed by sediment particles from the surface run-off or transported via 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 (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. Organisms living in close contact with sediments such as rooted macrophytes and microbial communities at the surface of the sediments (epipsammon) and within the sediments could thus experience a higher exposure to pesticides compared to exposure via the water phase. Such a presumed higher effect is supported by correlations found between sediment-bound chemicals and negatively affected macrophyte communities, while the phytoplankton community presumably remained unaffected (Machate et al., 2021).

Further, additional nutrient input via the sediment pathway may lead to a dominance by benthic algae (Jäger et al., 2017). On the other hand, submerged macrophytes can translocate pesticides from the sediment to the water phase (Diepens et al., 2014). In both cases, the negative effects on the entire ecosystems are rarely studied so far and the role of these two exposure pathways (sediment and water) on the occurrence of regime shifts remains unclear.

Pesticides and nutrients in the sediment may not only affect the rooted macrophytes but also the microbial community within the sediments (Barko et al., 1991). Since microbial activities are crucial for nutrient cycling, impairment of the sediment microbial community may in turn affect nutrient availability for rooted macrophytes. Microalgae that develop in the benthic boundary layer at the surface of the sediment, so called episammon, may play a critical role in the transfer of nutrients and pesticides between the sediment and the water column, thereby affecting the occurrence of regime shifts in the water column.

Climate change-induced elevated temperatures lower the threshold for ARO-induced regime shifts when exposed via the water phase (Polst et al., 2022). It is unclear, however, whether the same holds true when ARO is 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. 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, e.g. microbial degradation or accumulation of pesticides by organisms. At the same time this may be counter-acted by an increased metabolism and therefore higher growth rates of the 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 is not promoting shifts towards a turbid phytoplankton-dominated state compared to exposure via the water column, but affects growth of rooted macrophytes. 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. Methods

2.1 Microcosm setup

Microcosms were constructed as described in Polst et al. (2022). Cylindrical glass vases (height 40 cm, diameter 25 cm) were filled with 8 L Volvic® mineral water (Danone Waters Deutschland GmbH, Germany). 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, *Myriophyllum spicatum*, *Potamogeton perfoliatus* and *Elodea nuttallii*, were collected from presumably unimpacted sites 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 one 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 x 25 cm) were placed vertically on top of the sediment providing a surface for periphyton growth representing periphyton on macrophyte leaves. Natural phytoplankton (1×10^6 μm^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 (40W).

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 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 without any ARO compound, the solvent used for the pesticides (dimethylsulfoxid) in the ARO was applied to both, the sediment and the water phase of the same microcosm. The elevated temperature of Δ+4°C was selected based on Woolway et al (2021), who predicts such a rise during heatwaves in lakes.

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

	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 µg L^{-1}	0.75 µg L^{-1}	3.75 µg L^{-1}	22.5 µg L^{-1}	10.5 µg L^{-1}
ARO 4	9000 µg L^{-1}	3 µg L^{-1}	15 µg L^{-1}	90 µg L^{-1}	42 µg L^{-1}

2.3 Sampling

Available light and temperatures were logged on top of the sediment every five minutes (Hobo Pendant data logger, Onset Computer Cooperation, USA). Throughout the experiment, water samples were taken bi-weekly for pesticide analysis and weekly for nutrient analysis. To monitor the phytoplankton development over the course of the experiment, weekly phytoplankton samples (dry weight, pigments) were taken by filtration (0.7 µm glass-fibre filters, Labsolute, Germany). Four weeks after exposure of the microcosms all phototrophic compartments (macrophytes, phytoplankton, periphyton, 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 together for the microbial activity measurements and elemental C, N, P analysis. The upper layer (sand only, approx. 1 cm) was mixed together for pigment analysis of the periphyton developed at the sediment surface (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 and put on filters (0.7 µm) for dry weight (DW) measurement and pigment analysis (chl a).

2.4 Nutrient and pesticide analysis

Water samples for pesticide and nutrient analysis were filtered (0.22 µm, cellulose acetate filter, Labsolute, Germany). 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 porewater samples were analysed using an LTQ-OrbiTrap (Thermo Scientific, USA). Copper was analysed according to Vijayaraj et al. (2022). Dissolved inorganic nutrients (PO_4^{3-}, NO_3^-, NH_3, NH_4^+) were analysed photometrically according to DIN-EN-26777 (1993), DIN-EN-ISO 13395 (1996) and DIN-EN-ISO-6878 (2004). 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, Milan, Italy). Total P was oxidised 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

Filters with phytoplankton or periphyton biomass were extracted using 1 mL 90 % acetone and an ultrasonic bath at 55°C. Each filter was extracted twice and extracts were pooled. Pigments were analysed using a Bio-Tek Kontron HPLC with a diode array detector, according to the protocol of Woitke et al. (1994) and modified after Lips et al. (2022). For epipsammic community pigments, the collected sand from the sediment upper 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 000g, 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 mesocosm was collected and kept at -20°C until enzyme extraction. 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-vinylpolypyrrolidone, 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 (Fig. SI 1).

3.2 Concentrations of the agricultural run-off mixture

3.2.1 ARO concentrations in the water phase and in sediment porewater

Nitrate and pesticide concentrations in the water phase at the beginning of the experiment were within ±10 % of their nominal value (Table 2). Initial organic pesticide concentrations in the porewater of sediment-exposed 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 approx. 9.5 times higher, and tebuconazole was approx. 4 times higher. Yet the concentrations in the water column of sediment-exposed treatments were below detection limit. Concentrations of nitrate and copper in the sediment porewater could not be measured due to the low volume retrieved from sampling.

In the sediment-exposed treatments low amounts of nitrate translocated from the sediment to the water phase in the first week of the experiment (Fig. SI 2). 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 decreased with time (Fig. SI 3).

Table 2: ARO concentrations (mean ± sd, N-NO_3, Terbuthylazine, Pirimicarb, Tebuconazole, Copper) in the water phase and in the sediment pore water of the treatments undergoing exposure via the sediment one day after start of the exposure. (Terb = Terbuthylazine, Piri = Pirimicarb, Tebu = Tebuconazole, Cu = Copper; <dl = below detection limit, na = not available, Treatments: C = control, W = water-exposed, S = sediment-exposed, 1 = ARO 1, 4 = ARO 4)

			N-NO3 mg L^{-1}	Terb µg L^{-1}	Piri µg L^{-1}	Tebu µg L^{-1}	Cu 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	na	na	na	na
		Water phase	1.4±0.1	<dl	<dl	<dl	0.4±0.1
	S 4	Porewater	na	na	na	na	na
		Water phase	2.5±0.1	<dl	<dl	<dl	0.8±0.4

3.2.2 Pesticide concentrations in *Myriophyllum spicatum*

All pesticides were found in the apical part of *M. spicatum* but showed different accumulation between substances (SI Fig. 4): Terbuthylazine and Tebuconazole occurred at significantly higher concentrations in the water-exposed treatments than in sediment-exposed treatments. Exposure pathways did not lead to differences between treatments for pirimicarb.

3.3 Development of photoautotrophic compartments

At the lower ARO treatments at ambient temperatures macrophyte biomass was significantly lower in the water-exposed treatment, while no significant effect was found in the sediment-exposed treatment (Fig. 1). When exposed to higher ARO concentrations, both exposure treatments showed negative effects for macrophyte biomass. Phytoplankton showed a negative effect in the first week for the water-exposed treatment, but both treatments showed positive effects in the later weeks of the experiment. Additionally, episammon showed positive responses in the water-exposure treatments. No further effects on phototrophic biomass were observed in the sediment-exposed scenario. At heated conditions, only episammon showed a negative response to the lower ARO concentration, otherwise no effects were observed for this treatment. For the higher ARO concentration under warming, macrophyte biomass decreased in the water-exposed treatment and was not only significantly lower than the control but also lower than the biomass in the sediment-exposed treatment. Phytoplankton biomass decreased initially in the water-exposed treatment and showed an increase at the end of the experiment. The decline in macrophyte biomass parallel to an increase in phytoplankton biomass indicated a regime shift towards phytoplankton dominance.

The effects in the water-exposed treatments were similarly driven by the three tested macrophyte species, while in the sediment-exposed treatments only *P. perfoliatus* showed a significant decline in dry weight (see Fig. SI 5). The root:shoot (length) ratio of all three macrophyte species did not show significant changes for any treatment (data not shown).

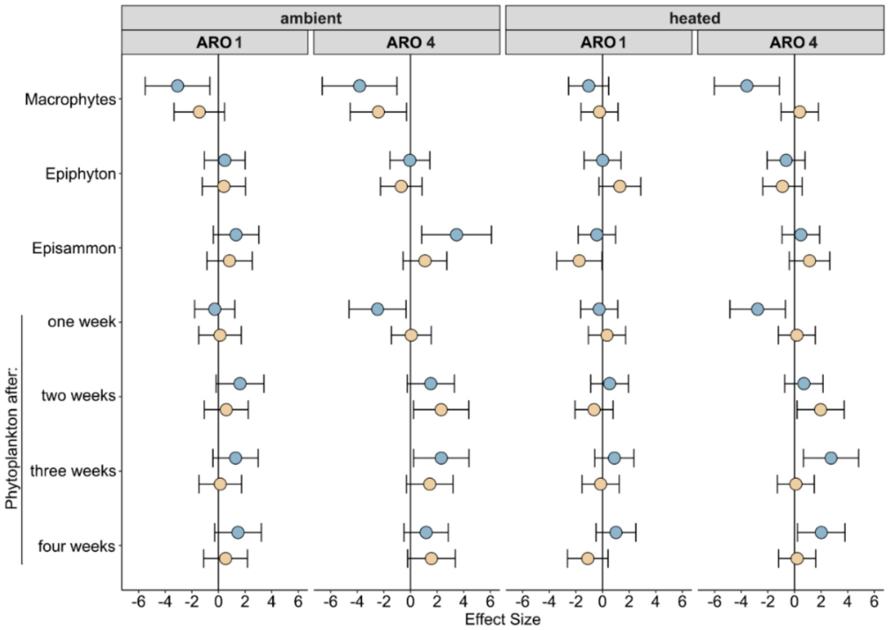


Figure 1: Effect sizes (mean ± sd; blue dots = exposure via water column, brown dots = exposure via sediment) for phototrophic biomass (macrophyte, periphyton, and episammon at the end of the experiment and phytoplankton over the course of the four weeks after exposure).

3.4 Microbial functions and nutrient cycling in sediments

Exposure pathways led to only few differences in elemental content of macroelements and exoenzymatic activity in the sediment layer (Fig. 2). While carbon and nitrogen content in sediments showed no exposure related effect, the phosphate content increased significantly at the highest ARO concentration in the sediment-exposed treatments.

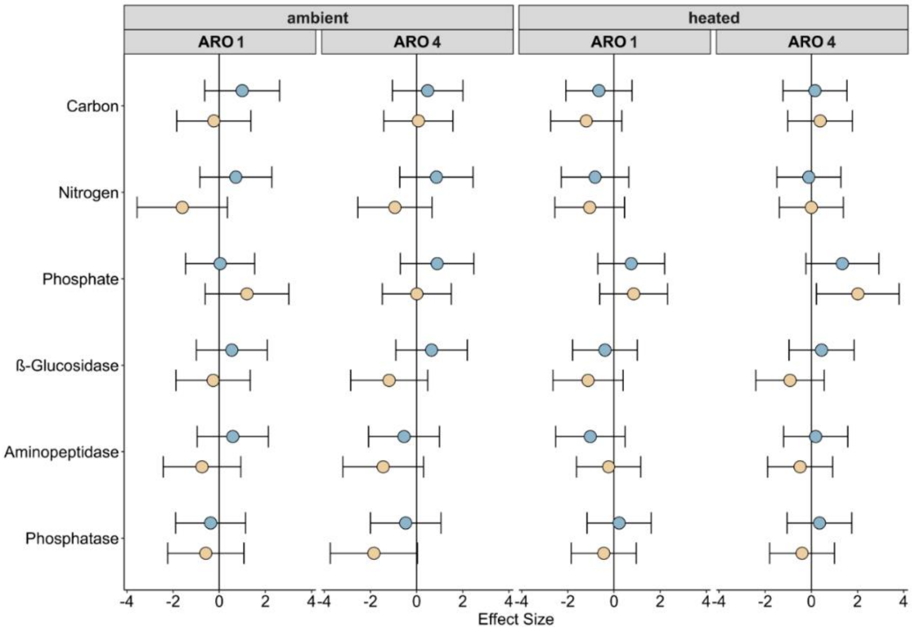


Figure 2: Effect sizes (mean ± sd; blue dots = exposure via water column, brown dots = exposure via sediment) for the elemental composition of the sediment (content in carbon, nitrogen and phosphorus) and exoenzymatic activity (ß-glucosidase, aminopeptidase, phosphatase).

4. Discussion

Our results demonstrate that exposure pathways of chemical stressors matter for their effects on primary producers and sediment-nutrient dynamics in shallow lakes and ponds subjected to ARO. In general, our study confirmed that exposure to agricultural run-off containing nitrate and pesticides via the water phase can result in regime shifts towards phytoplankton dominance. Confirming our expectation, ARO exposure via sediments did not change the dominance of primary producer groups and, contrary to our expectation and previous work (Polst et al., 2022), elevated temperatures had only minor effects on the biomasses of phototrophic organisms. However, it should be noted that under *in-situ* conditions, several activities (e.g. feeding, burrowing, etc.) of benthic invertebrates or benthivorous fish may also have relevant effects on bioturbation and on the direction and magnitude of the redistribution of organic matter pesticides and nutrients (Bundschuh et al., 2016; Hölker et al., 2015; Zhang et al., 2022).

4.1 Fate of pesticides

When ARO was added to the sediments, the three organic pesticides partly leached into the water column towards the end of the experiment, thus the sediment acted as a source of pollution in the water phase. The pesticide concentrations in the sediment pore water of the ARO mixture decreased at a higher rate compared to pesticides in the water column. Likely, pesticides were absorbed to sediment particles at different magnitude related to their partitioning coefficient (log $K_{OW,}$ see table 1), resulting in pirimicarb showing the highest concentration in the porewater and the highest translocation to the water phase due to its hydrophilic properties.

Besides pesticide occurrence in the sediment pore water and the water phase, the three pesticides were found in apical shoots of *M. spicatum* at the end of the experiment. Accumulation 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 (Diepens et al., 2014). The high pirimicarb concentrations in the water of the sediment-exposed treatments indicates a translocation of pesticides from the sediment to the water column followed by an uptake in *M. spicatum* via the water column. This is in line with Diepens et al. (2014) who found translocation from pore water into the water column and subsequent uptake by macrophytes is likely the primary pathway for pesticides, with uptake via the roots and transport to the shoots being secondary. However, translocation and uptake pathway depend on hydrophobicity of the pesticides; the low internal concentrations of the herbicide terbuthylazine likely contributed to the lack of effects on *M. spicatum* in the sediment-exposed treatments.

4.2 Nutrient dynamics

The N-content of the sediment at the end of the experiment was not affected by the addition of ARO to the sediment or to the water while ARO included nitrate. This result indicates a fast leaching of nitrate from the sediment into the water column in the sediment exposed treatments. This leaching 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, nutrients in both exposure pathways decreased rapidly in the water and were probably converted into biomass by either phytoplankton or benthic microalgae. Microbial activities in the sediment related to nutrient cycling were not negatively affected by ARO exposure, we thus cannot confirm our second hypothesis. Further, elevated temperatures did not lead to higher resuspension of ARO from the sediment into the water as expected in the third hypothesis.

Despite a higher nitrate loading, no significant increase in epipsammic biomass with sediment exposure was observed. However other factors, e.g. negative effects due to herbicide exposure, may have limited its development. Higher level of nitrates found in sediments indicate a negative effect on the uptake of phosphorus in the sediments by macrophytes or a lower release to the water column.

4.3 Exposure related regime shifts

Regime shifts induced by ARO, indicated by a lower macrophyte growth and an increased phytoplankton or periphyton biomass, were only observed in the water-exposed treatments supporting findings of other microcosm studies testing the same ARO mixture (Allen et al., 2021; Polst et al., 2022; Vijayaraj et al., 2022). The decline in macrophyte biomass was not driven by all macrophyte species equally and highlights that the response in field situations can depend on the dominant species (Cedergreen et al., 2004; Wu et al., 2021).

The observed effects on macrophytes can be caused by direct toxicity of the pesticides, indirectly by shading by a nitrate-induced phytoplankton bloom or an interaction of both as found by Wendt-Rasch et al. (2004) in a microcosm experiment including *M. spicatum*. The concentration of pesticides used in our study was relatively low compared to EC_{50} values for macrophytes (Giddings et al., 2013), and no clear direct toxic effect on macrophytes have been identified in previous studies using similar concentrations of the same pesticides cocktail (Allen et al., 2021; Vijayaraj et al., 2022). Consequently, the higher phytoplankton density resulting in reduced light availability more likely caused the decline in macrophyte biomass.

Exposure of ARO via the sediment only affected macrophyte growth and not microalgae supporting findings by Machate et al. (2021). Pesticides in the sediment porewater can thus negatively affect macrophyte growth through direct effects unrelated to shading by microalgae. Yet, species-specific sensitives towards pesticides are again important as two of the three macrophyte species were not affected in their growth after four weeks of ARO exposure via the sediment.

Elevated temperatures negated previous negative effects of ARO on macrophytes via sediment exposure, which was not found in the water exposed treatments. We expected convergence of effects on both treatments (third hypothesis), due higher ARO remobilization from the sediment. Instead of regime shifts in both exposure treatments, effects of sediment-exposed ARO were negated and elevated temperatures led to macrophyte dominated clear water states in both exposure treatments. Higher temperatures enhance macrophyte growth as long as they do not exceed the species-specific optimal temperature (Feuchtmayr et al., 2009; Hansson et al., 2020; Zhang et al., 2019). Apparently, the negative effect of pesticides released via the sediment are counteracted by an increased metabolism and growth of macrophytes, which has also been described by Vijayaraj et al. (2022). Longer exposure of ARO via the sediment than in our study may eventually also lead to a loss of macrophytes.

5. Conclusion

Small and shallow aquatic ecosystems fulfil an important ecological role in agricultural landscapes, but are exposed to high nutrient and pesticide loadings via water and sediments (Machate et al., 2021; Wijewardene et al., 2021). Our short-term laboratory experiments indicate that agricultural run-off associated to the water phase is more likely to lead to regime shifts with phytoplankton dominance than sediment exposure. However, longer exposure periods and the inclusion of higher trophic levels interacting with sediments such as macroinvertebrates and fish may change these results and require further studies. Ultimately, exposure pathways, translocation of pesticides and interacting effects of co-occurring multiple stressors need to be considered when designing mitigation measures to protect these vulnerably, but frequent aquatic systems in agricultural landscapes.

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Bastian 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.

Supplemental Information

Table of contents

1. Light availability

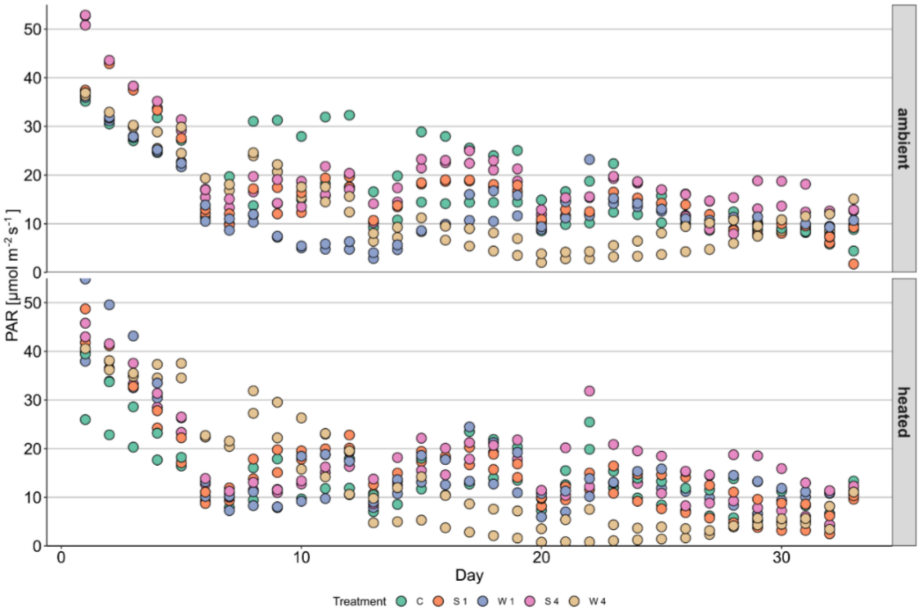

Figure SI 1: Light availability (daily average per microcosm; two microcosms per treatment) at the bottom of the microcosm throughout the experiment measured with Hobo light data loggers. Treatments: green = control, orange = S 1, blue = W 1, pink = S 4, yellow = W 4.

2. Nitrate concentrations

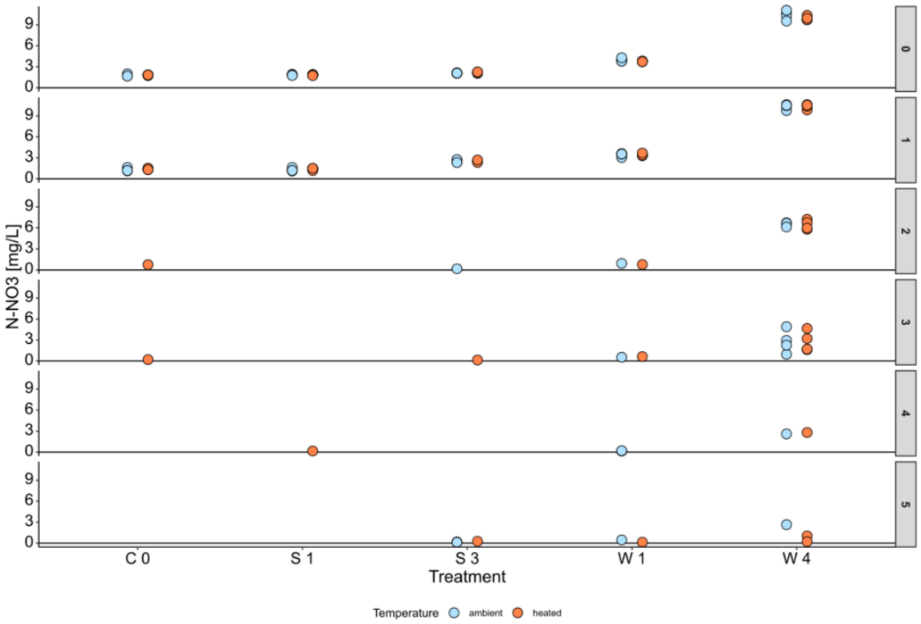

Figure SI 2: N-NO$_3$ concentrations in the water column for ambient (blue dots) and heated (red dots) conditions at different time points through the experiment (T0 = day of treatment application, T1 = within the first week, Tx = xth week, T5 = during final sampling).

3. Pesticide concentrations in the water column and the sediment porewater

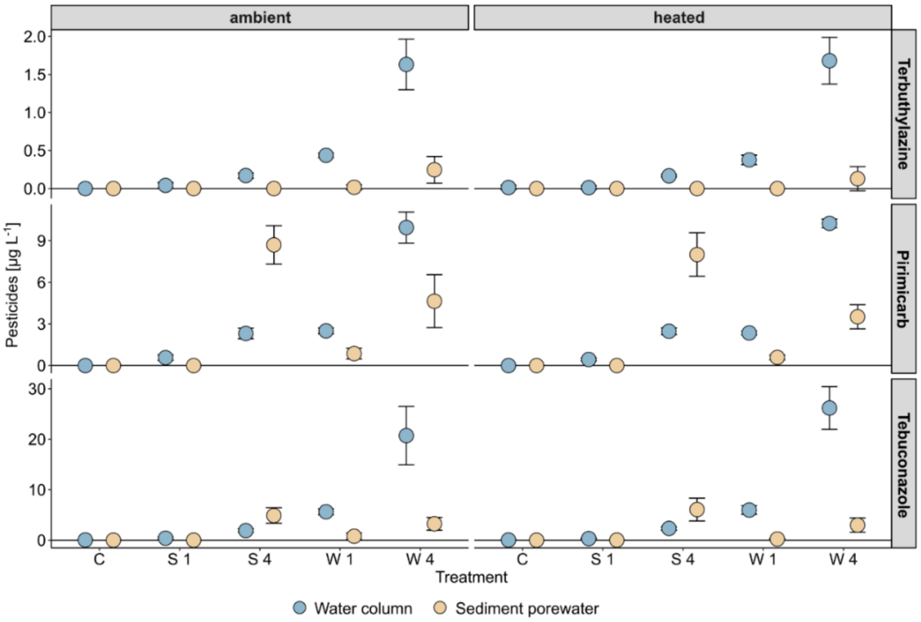

Figure Si 3: Organic pesticide concentrations at the end of the experiment measured in the water column (blue) and sediment porewater (brown).

4. Pesticide concentrations in M. spicatum

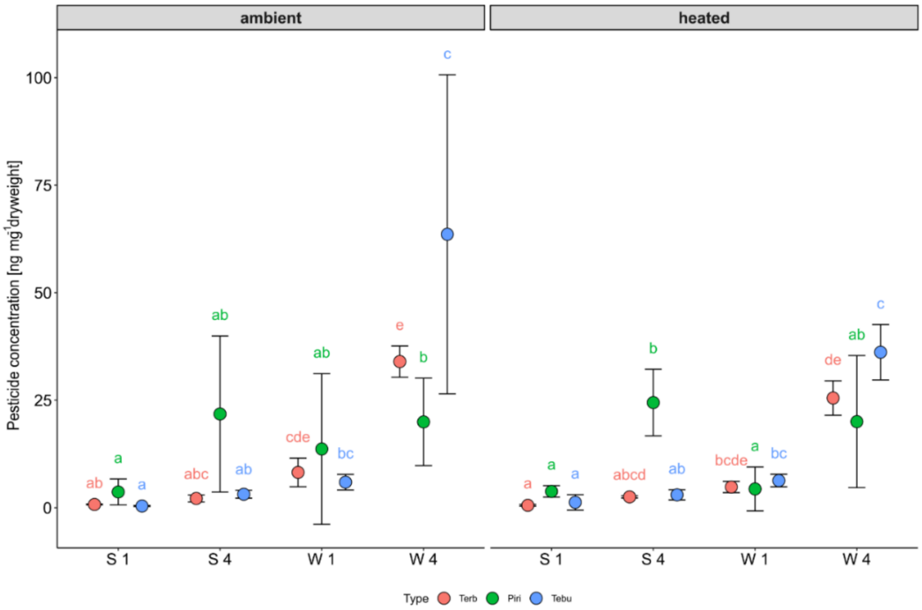

Figure SI 4: Pesticide concentrations (red = terbuthylazine, green = pirimicarb, blue = tebuconazole) in the apical 10 cm of *Myriophyllum spicatum* at the end of the experiment. Plant biomass from the control treatments were used to test the recovery of pesticides of the extraction method and are therefore 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 colors.

5. Effects on macrophyte biomass of the individual macrophyte species

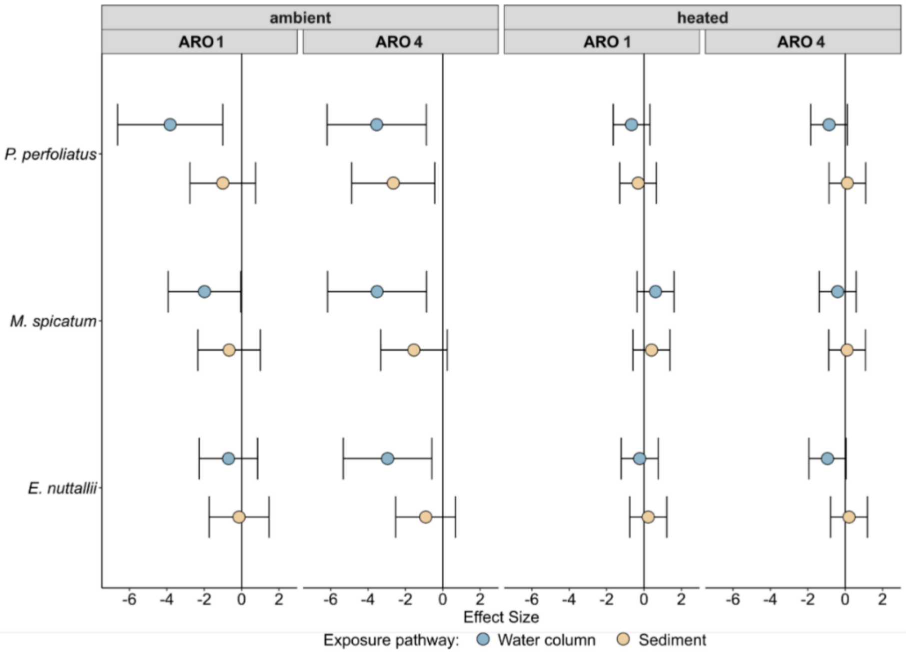

Figure SI 5: Effect sizes (Hedges' g) for effects on the three macrophyte species *P. perfoliatus*, *M. spicatum* and *E. nuttallii*.

5: Evaluating multiple stressor effects on benthic-pelagic freshwater communities in systems of different complexities: Challenges in upscaling

Vinita Vijayaraj, Nora Kipferler, Herwig Stibor, Joey Allen, Franz Hölker, Martin Levaile, Joséphine Leflaive, Gregoria Alejandro López Moreira Mazacotte, **Bastian H. Polst**, Mechthild Schmitt-Jansen, Sabine Hilt, Elisabeth M. Gross

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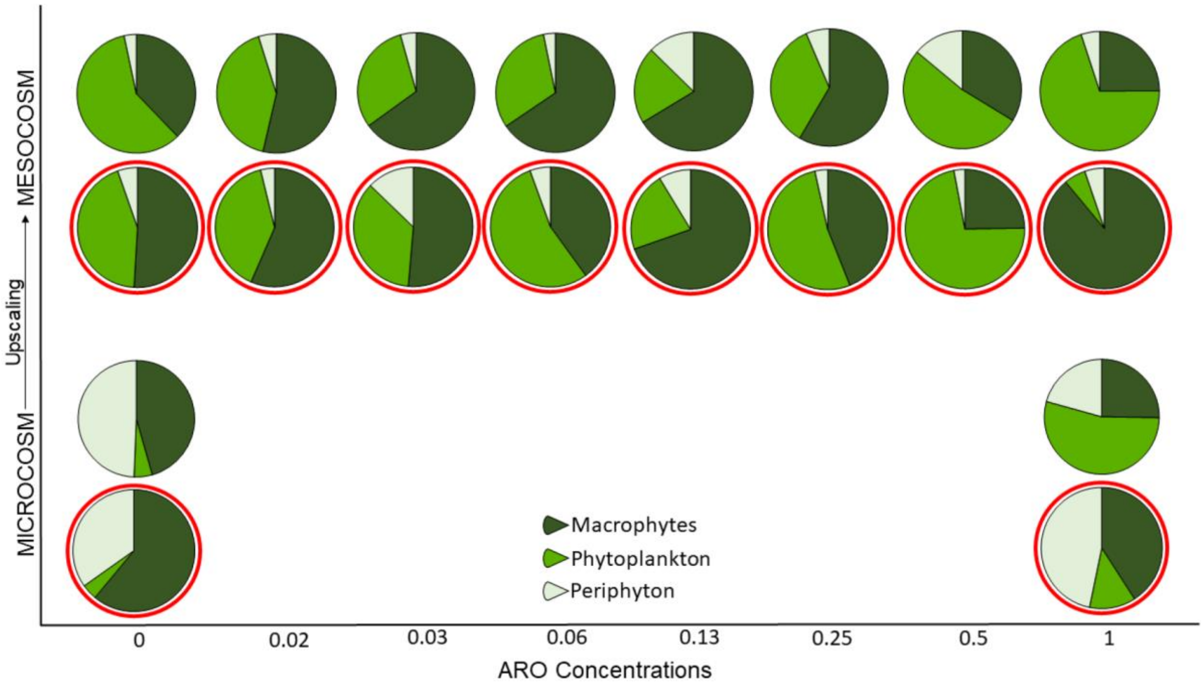

Figure 17 | Key result of the following *Trophic Upscaling* chapter showing the share of phototrophic groups in the micro- and the mesocosms. The red circle indicate treatments with elevated temperature.

water

MDPI

Article

Evaluating Multiple Stressor Effects on Benthic–Pelagic Freshwater Communities in Systems of Different Complexities: Challenges in Upscaling

Vinita Vijayaraj [1,2,†], Nora Kipferler [3,†], Herwig Stibor [3], Joey Allen [1,4], Franz Hölker [5], Martin Laviale [1,2], Joséphine Leflaive [4], Gregorio Alejandro López Moreira Mazacotte [5], Bastian Herbert Polst [6], Mechthild Schmitt-Jansen [6], Sabine Hilt [5] and Elisabeth Maria Gross [1,2,*]

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Abstract: Upscaling of ecological effects from indoor microcosms to outdoor mesocosms bridging the gap between controlled laboratory conditions and highly complex natural environments poses several challenges: typical standard water types used in laboratory experiments are not feasible in large outdoor experiments. Additionally, moving from the micro- to meso-scale, biodiversity is enhanced. We performed an indoor microcosm experiment to determine the effects of agricultural run-off (ARO) on a defined benthic–pelagic community comprising primary producers and primary consumers, exposed to ambient summer temperature and +3.5 °C. Treatments were replicated in two water types (standard Volvic and Munich well water). We then scaled up to outdoor mesocosms using an ARO concentration gradient and +3 °C warming above ambient temperature, using Munich well water. We included the same benthic macroorganisms but more complex periphyton and plankton communities. All the functional groups were affected by stressors in the microcosms, and a shift from macrophyte to phytoplankton dominance was observed. While effects were present, they were less pronounced in the mesocosms, where a higher biodiversity may have modified the responses of the system to the stressors. The stressor effects observed in controlled experiments may thus be masked in more complex outdoor experiments, but should not be interpreted as "no effects".

Keywords: microcosms; mesocosms; community complexity; stressor interactions; biotic interactions; copper; terbuthylazine; pirimicarb; tebuconazole; climate warming

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1. Introduction

Alternative stable states between phytoplankton and macrophytes commonly occur in shallow lakes, and state shifts are primarily driven by eutrophication [1]. The likelihood for such shifts may be enhanced by warming, e.g., through an increased release of nutrients from the sediment [2]. While the focus of many eutrophication studies in shallow lakes has been on phosphorus, recent studies show that high nitrate concentrations may also facilitate to regime shifts [3–5]. High nutrient input into lakes occurs after heavy rainfall events through surface run-off from agricultural sites also containing pesticides. The ecotoxicological effects of these pesticides are well studied at the organismic level, but the community- and ecosystem-level effects are less understood. A combination of nutrient and pesticide stressors may further facilitate the shift to turbid conditions.

With the growing use of pesticides and fertilisers, and rising global temperatures, identifying safety margins to preserve the stability of the macrophyte-dominated state in these systems exposed to such multiple stressors is essential. Starting with laboratory-controlled microcosms comprising less complex benthic–pelagic communities and upscaling to more complex and variable communities in outdoor mesocosms might allow for the identification of stressor patterns or effects as they would occur in natural ecosystems, even in the presence of confounding factors.

Indoor microcosms allow a better control of environmental factors and can be set up with key functional groups representative of shallow lake benthic–pelagic communities, such as macrophytes, phytoplankton, periphyton, benthic and pelagic filter feeders and grazers. They allow the use of organisms common in standardised ecotoxicity tests, such as *Daphnia* [6,7], algae and the OECD test plant *Myriophyllum spicatum* [8], all typically playing key roles in natural systems. Most importantly, microcosms can be designed to couple benthic and pelagic organisms. Upscaling to larger outdoor mesocosms will further enable the inclusion of natural environmental factors, such as irradiance and temperature fluctuations, more diverse communities with more species representative of each functional group and/or ecosystem type and more trophic levels.

Upscaling, however, can pose several challenges due to the size of experimental units and natural variability in abiotic and biotic factors. The chemistry of the water used in the experimental set-up is important [9,10], as is the technical availability in terms of costs and logistics. In controlled laboratory experiments, defined culture media or widely available bottled natural mineral waters (e.g., Volvic®) are often used, allowing for the comparison of results among research groups [11,12]. However, using this for large outdoor mesocosms at the cubic metre scale is not feasible. Most mesocosms are usually filled with surrounding lake/sea water or tap/groundwater, depending on availability [13–16]. While these waters vary in their physico-chemical properties, it may still be reasonable to use them in mesocosms, provided precautions are made based on the organisms' needs. For example, adapting the concentration and ratio of initial macronutrients, conductivity range, alkalinity, pH and dissolved organic and inorganic carbon of the water at the start of the experiment may allow optimum conditions and better comparability between the experiments. In addition to the central parameter "water type", other abiotic parameters, such as photoperiod, temperature, pH, dissolved oxygen and light intensities vary over time [17], making them less controllable than laboratory microcosms. Such varying natural abiotic conditions can play an important role in influencing the response of organisms to stressors, and should be well monitored during the experiments [18–20].

Most studied mesocosms also suffer from large temporal and spatial variations between replicates, in some cases, with more than 50% of the variation being observed on biological variables [21]. By increasing community complexity in mesocosms, several direct and indirect effects may modify the response of the system to stressors compared with microcosms where biotic interactions are less complex [22,23]. Culturing enough organisms for stocking large mesocosms is challenging and sometimes impossible. To solve this issue, mesocosm studies usually incorporate organisms from natural communities, e.g., by using natural plankton or benthic communities from field sites versus relying on specific cultured organisms in laboratory experiments. While this increases the complexity of the community, it may also be the aim of the study, as a higher biodiversity allows more closely mimicking stressor effects in a real-world habitat.

Outdoor experiments are also prone to species invasions into the experimental units. These may not always be easy to control, and in some cases may lead to new trophic levels, which may reverse the overall responses of the system to nutrient enrichment [24]. Some species invasions can be prevented using specific measures (e.g., netting against birds), but open outdoor mesocosms cannot be easily protected from invasions by smaller organisms, such as aquatic insects without interfering, e.g., with light climate and gas exchange. Therefore, decision-making on which invasions can be informative and allowed, and which might be disruptive to the data analyses is fundamental to answering specific research questions.

This article focuses on some of the challenges of upscaling research on the effects of multiple stressors on shallow aquatic systems by combining small-scale laboratory studies to large-scale outdoor experiments. Microcosms were designed to analyse the effects of ARO and temperature on a predefined less complex and a priori assembled benthic–pelagic community. Mesocosms were planned to be more complex, with the aim of studying the responses of natural and diverse communities to a gradual increase in ARO associated with elevated temperature. Microcosms were run with a well-defined mineral water (Volvic water) whereas the large water volume needed for mesocosms could only be met by using Munich well water available at the site where the mesocosm experiment was conducted (Ludwig Maximilian University of Munich (LMU), Martinsried, Germany). To compare the effects of using either standard Volvic or Munich well water, the controlled microcosm experiments were performed with both water sources. We hypothesised that:

1. Water type will not modify effects of the stressors;
2. Response of model (laboratory) communities to the stressors can be mirrored in more complex field (mesocosm) communities;
3. A gradient design will allow for the detection of concentration-dependent community effects in more complex systems.

2. Materials and Methods

2.1. Microcosm Experiment

2.1.1. Set-Up and Design

We performed experiments beginning with smaller controlled indoor microcosms each with a volume of 8 L exposed to ARO and warming. Aquatic organisms were selected as representative of fishless ponds, and included two trophic levels (primary producers and consumers) comprising key functional groups: macrophytes, phytoplankton and periphyton as the three major groups of primary producers in aquatic systems, *D. magna* as representative of pelagic herbivore filter feeders (food source: phytoplankton), and *L. stagnalis* as representative of benthic grazers (food source: periphyton). The macrophyte community consisted of three submerged macrophytes, *Myriophyllum spicatum*, *Potamogeton perfoliatus*, and *Elodea nuttallii*. The microalgae community was based on eight cultured strains of periphyton or phytoplankton similar to Allen et al. [22], except *Uronema confervicolum* and *Gomphonema parvulum,* which did not grow in our cultures. The second trophic level included three primary consumers: snails (*Lymnaea stagnalis*) feeding on periphyton and sometimes macrophytes, and mussels (*Dreissena polymorpha*) and zooplankton (*Daphnia magna*) feeding on phytoplankton. Half of the microcosms were exposed to ARO, comprising copper as an inorganic fungicide, three organic pesticides (a herbicide, an insecticide and a fungicide) and nitrate (as KNO_3), and the other half were controls. The chosen pesticides are widely used in European agriculture, and concentrations of all chemicals are based on sensitivity data, as well as background environmental concentrations. Further details of the ARO can be found in Table S1 and Allen et al. [22]. The treatments were replicated at two temperatures, aiming for a +4 °C increase above ambient temperature in the heated microcosms, as projected by the IPCC RCP 8.5.

To determine whether the water type can affect the community response to applied stressors (temperature and ARO), we performed a microcosm experiment using a full-factorial design with 2 water types × 2 pesticide levels × 2 temperatures × 5 replicates, for a total of 40 microcosms. We used two water types: Volvic water (Vw), as a frequently used standard in experimental research, and Munich well water (Mw), which was our choice in the mesocosm experiments. Our treatments were the controls in Vw (VCON) and Mw (MCON), and those exposed to ARO in Vw (VARO) and Mw (MARO), each replicated at two temperatures. The chemistry of both water types was analysed using standard methods before the experiment. This included alkalinity (Gran titration), major anions (nitrate, nitrite, orthophosphate, chloride, sulphate; ion chromatography, Dionex ICS 1100, Thermo Fisher Scientific France, Illkirch-Graffenstaden, France), ammonium (spectrophotometry) and alkali elements (Ca, K, Mg, Na; flame ionisation, Thermo Scientific ICE 3300, Thermo Fisher Scientific France, Illkirch-Graffenstaden, France).

The microcosms comprised a crystallizing dish insert (height 8 cm, Ø 15 cm) filled with a layer of sediment according to the OECD TG 239 [8], but by replacing inorganic nutrient salts with nettle powder (0.5% w/w). Each dish was placed within a glass cylinder (height 40 cm, Ø 19 cm; Sandra Rich GmbH, Ebernhahn, Germany) filled with 8 L of the respective water. The microcosms were then distributed evenly into 4 glass tanks, consisting of circulating, temperature controlled water baths, 2 each at 22 °C and 26 °C. However, the average minimum and maximum temperatures reached with the heating system were 21.1 °C ± 0.2 °C and 24.6 °C ± 0.3 °C, for a difference of ~3.5 °C between the 2 temperature treatments.

All species were acclimated to experimental conditions for at least 4 weeks and sorted for size before addition to the microcosms, to ensure homogeneous distribution among the treatments. The microalgae were cultured in BG11 [25] or WC [26] medium and adapted to Vw before the start of the experiment. Four polypropylene sheets measuring 29.7 cm × 2.6 cm length × width were hung vertically into each microcosm for follow-up of periphyton development (cf. Section 2.2.2). Before the microalgae were added to the microcosms, the Mw treatments were fertilised with phosphorus to achieve the Redfield molar ratio (16:1 N:P) similar to that present in Vw. Similar biovolumes of both the periphyton and phytoplankton were added to the microcosms six days before the start (T-6). Two days later, two 10 cm shoots of each macrophyte species were planted into the sediment (T-4). Twenty *Daphnia* neonates, one snail and three mussels per microcosm were added on day T-2. The ARO was added at T0, and at the same time, the temperature of half the treatments was set at 26 °C. The microcosms were exposed to a 16:8 h day:night cycle with irradiance at an average of 76.01 ± 7.9 μmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR) at the water surface (ToLEDo LED fluorescent tubes, cool white, 150 cm, 27 W, Sylvania; RS Components, Beauvais Oise, France).

2.1.2. Sampling and Measured Parameters

Weekly measures of pH, conductivity and oxygen saturation of the water were made using a multi-parameter analyser (WTW Multiline 3410; Champagne-au-Mont-d'Or, France). Dissolved inorganic nutrients were measured after inoculation with organisms, but before exposure.

At the end of the experiment (4 weeks), water was first sampled for chemistry, followed by plankton and periphyton, and then the benthic consumers. The macrophytes were sampled at the end to ensure minimum disruption to the system.

During the final sampling, the dissolved inorganic nutrients were again measured in all the microcosms. The optical density of the water at 663 nm, used as a proxy for the development of phytoplankton over time, was measured weekly with a Varian Cary® UV-VIS spectrophotometer (Varian GmbH, Frankfurt, Germany).

Depending on the final density of phytoplankton, between 40 and 100 mL per sample was filtered (25 mm GF/F filters, 0.7 μm pore size, Whatman France Sarl, Versailles, France) for the analysis of carbon. The periphyton on the polypropylene strips was brushed gently into 20 mL Vw. Pellets were homogenised, centrifuged, frozen and lyophilised, and the dry powder used for carbon analyses. Photosynthetic pigments of microalgae were analysed by HPLC-DAD (high performance liquid chromatography-diode array detector) (UHPLC Ultimate 3000 Rs THERMO; Thermo Fisher Scientific France, Illkirch-Graffenstaden, France).

Weekly *Daphnia* counts were made by collecting 50 mL of water after gentle stirring of the water column. This number was extrapolated to the total volume per microcosm.

The counted *Daphnia* were returned to the microcosms. At the end of the experiment, all *Daphnia* were collected. Their biomass was estimated from length measures, obtained using a numerical microscope (VHX-6000; Keyence; Bois-Colombes, France): $B = 0.01 \times L^{2.62}$, where B represents biomass in mg and L = length in mm [27].

The snails and mussels were removed and their lengths measured.

The macrophytes were removed, including the roots, rinsed and separated into aboveground and belowground parts. The carbon content of the apical 10 cm dry plant shoot was measured. The carbon content of primary producers was measured using a CHNS elemental analyser (Carlo-ERBA Na 2100 CE; Carlo Erba, Val de Reuil, France).

2.1.3. Data Analysis

A three-way ANOVA was performed using R (v4.1.0; [28]) to test the individual and combined effects of water type, ARO and temperature on all end points. Residuals were first tested for normality and homoscedasticity using the Shapiro–Wilk test and the Bartlett test, respectively. Log or square root transformations were performed if the data did not fit these assumptions. When the data could not be normalised, a Kruskal–Wallis test by ranks was applied. Significance was considered at $p < 0.05$. The Hedges' g (Hedges, 1981) were also calculated from the *F*-statistics derived from the three-way ANOVA to determine the size of individual and interaction effects on the biomass of primary producer groups, and on the length or biomass of consumers (using the esc package in R; [29]). To determine significant ARO, water or temperature effects on the distribution of the three primary producer groups, a PERMANOVA was performed using the vegan package [30] in R, with proportions (values from 0 to 1) of macrophyte, phytoplankton and periphyton carbon content as the dependent variables. Principal components analyses (PCAs) were performed using the vegan [30] and factoextra packages [31] in R to determine any strong patterns in the data.

2.2. *Mesocosms*

2.2.1. Set-Up and Design

Following the microcosm experiment, we scaled up to larger outdoor mesocosms set up at LMU (48°6′31.961″ N 11°27′26.896″ E) with a total 16 enclosures and volumes of 640 L each using Munich well water and exposed to a gradient of ARO to determine the concentration-dependent effects on the primary producers. See Figure 1 for a comparison of the design of the indoor microcosms and outdoor mesocosms. We aimed to have a similar trophic structure as in the microcosms (Table S2), with two trophic levels, but with the higher biodiversity (at both taxonomic and functional levels) provided by natural periphyton and plankton communities (see Table S3 for a comparison of morphotypes between micro- and mesocosms). We included the same three macrophyte species, snails and mussels, and obtained the periphyton and plankton community inoculum from nearby eutrophic and mesotrophic lakes (Lake Bannsee, 47°57′52.4″ N 12°26′25.1″ E, and Lake Klostersee, 47°58′21.5″ N 12°27′25.6″ E). Since the mussels had a high mortality rate in both experiments, irrespective of treatment type, they are not discussed further. The highest ARO concentration was set as similar to the microcosms, and then diluted in 6 steps at a 1:1 dilution factor (Table S1). This gradient was applied to two series of mesocosms differing in temperature (eight at ambient environmental and eight at a +3 °C increase, including daily temperature fluctuations). The +3 °C difference was chosen to stay similar to the actual temperature difference obtained in the microcosms (+3.5 °C). For both experiments, thrice weekly, macronutrients were added at the Redfield ratio (16 μmol L^{-1} N and 1 μmol L^{-1} P as KNO_3 and KH_2PO_4, respectively) to ensure the growth of the primary producers.

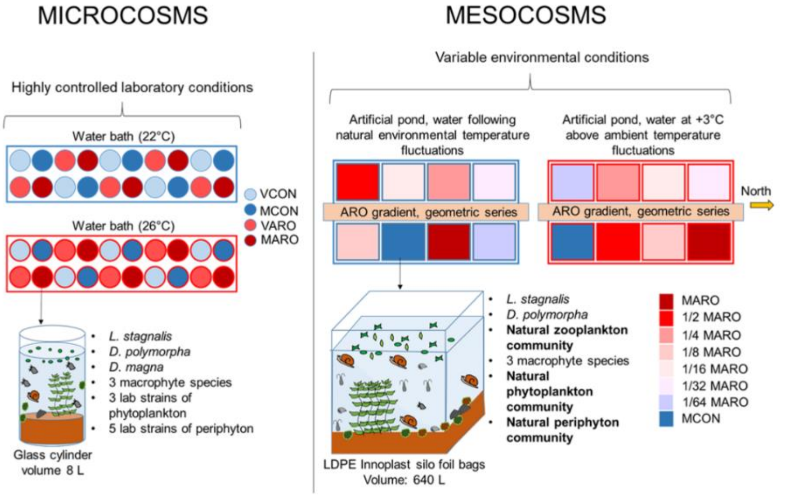

Figure 1. Comparison of the design between the indoor controlled microcosms and the outdoor variable mesocosms. The concentrations of the pesticides and nitrate comprising the agricultural runoff (ARO) in the microcosms were comparable to the highest ARO in the mesocosms. VCON = Volvic water control, MCON = Munich water control, VARO = Volvic water ARO and MARO = Munich water ARO.

Each of the sixteen enclosures consisted of an inner watertight layer (Innoplast silo foil; BayWa, Munich, Germany) and an outer weight-bearing layer. They were suspended from wooden beams in two concrete ponds. This way, all enclosures in the same pond were kept at the same temperature and the heated enclosures could be heated from the outside using two industrial heating elements (48 KW, ISA-Heinrich-Industrietechnik, Falkensee, Germany). Each mesocosm contained 640 L well water resulting in a water column of 70 cm above a sediment layer of 10 cm. Mesocosms stayed without cover and open to full solar radiation, precipitation and evaporation. The average temperatures throughout the experiment ranged from 16 to 22 °C, with a peak of 27 °C in the ambient mesocosms and from 19 to 25 °C with a peak of 31 °C in the heated. The difference of 3 °C remained consistent between the temperature treatments. The average irradiance ranged from 300 to 2000 µmol photons m^{-2} s^{-1}.

Before starting the experimental manipulations (T0), all the organisms were given time to establish in the new environment. Setup started one month before (T-31) with the deployment of the mesocosms. Each one was filled with 80 L of sediment consisting of two layers. An even mixture of sand and soil constituted the bottom layer. This was covered by another layer of sand (Spielsand 0–1 mm and Fortunat Humus 0/5 sieved, Bernhard Glück Kies-Sand-Hartsteinsplitt GmbH, Gräfelfing, Germany). Without agitating the sediment layers, 590 L of well water was pumped into each mesocosm.

A natural phytoplankton community was introduced by adding 50 L of water from the eutrophic lake Bannsee to each mesocosm on the next day. Natural periphyton collected from Lake Klostersee and resuspended in lake water was added to all mesocosms. An initial pulse of 9.8 µmol L^{-1} P to adjust the N:P ratio in Mw was added.

On days 16 and 15, the macrophytes were planted. Ten stems of *M. spicatum* and *P. perfoliatus* and fifteen stems of *E. nuttallii* were planted as three distinct patches at the same location in each mesocosm. Along the middle north–south axis, 12 transparent polypropylene strips as support for periphyton (2.5 × 60 cm) were hung in each mesocosm. Snails and mussels were released to the mesocosms on day 4 after homogenisation for size.

Zooplankton was collected from Lake Klostersee with a 250 µm net and introduced into the mesocosms on day 2.

2.2.2. Sampling and Measured Parameters

Integrated water samples were taken with a tube sampler (1 L). The phytoplankton chlorophyll-*a* concentration was measured with a multispectral fluorometer (AlgaeLab-Analyser, bbe Moldaenke GmbH, Schwentinental, Germany). The phytoplankton carbon and nitrogen content were measured with an elemental analyser (varioMICRO Cube, Elementar Analysensysteme GmbH, Langenselbold, Germany). For continuous light intensity and temperature measurements, HOBOs (HOBO MX2202Pendant, Onset Computer Corporation, Bourne MA, USA) were placed on top of the sediment in the middle of the macrophyte-free quarter.

Periphyton was sampled bi-weekly for chl-a measurements (adapted from Woitke et al. [32] and described in Schmitt-Jansen and Altenburger [33]), and at the end of the experiment, the dry weights were measured. The carbon equivalents were estimated as 60% dry weight based on the average percentages calculated in a previous experiment (VV, unpublished).

Snails were collected before the mesocosms were dismantled, and their lengths measured.

After eight weeks of exposure, the mesocosms were dismantled one by one. The whole water column was passed through a 250 µm net and zooplankton and insect larvae were collected. Additionally, animals clinging to plants and the mesocosm wall or the sediment were collected. All the samples were fixed in 96% ethanol. Individual species groups were counted using a stereo microscope. The samples were then dried and weighed.

Once all the water was removed from the mesocosms, all the macrophytes were taken out by gently releasing the roots from the sediment until the plants were free but intact. The total dry biomass per species and mesocosm was determined after gently rinsing and removing debris and/or insects, and drying for at least 48 h at 80 °C. The carbon content was determined similar to the microcosms.

For both the experiments, copper was analysed in LIEC, Metz, France, by atomic absorption spectroscopy (Varian SpectrAA 800 Zeeman; Thermo Fisher Scientific France, Illkirch-Graffenstaden, France) and the organic pesticides were analysed in UFZ, Leipzig, Germany, after filtering water samples (0.22 µm PVDF syringe filters), by liquid chromatography–mass spectrometry using an LTQ Orbitrap XL (Thermo Fisher Scientific, Karlsruhe, Germany).

2.2.3. Data Analysis

Simple linear and quadratic regressions were tested on the primary producers using R [28] to identify any relationships between the stressors and their biomass.

2.3. Comparison of the Primary Producer Community Structure in Micro- and Mesocosms

The primary producer community structures in the micro- and mesocosms were compared using the biomasses of each primary producer group expressed as total carbon. For the phytoplankton, the measured carbon was extrapolated to the total volume per microcosm (8 L) or mesocosm (640 L) to obtain total carbon. For the periphyton, the carbon values were roughly estimated by extrapolating to the surface area of the polystyrene strips plus inner surface of either the microcosm glass walls or the mesocosm enclosure walls. For the macrophytes, the total carbon was calculated from the total macrophyte aboveground biomass per micro- and mesocosm.

3. Results

3.1. Microcosm-Effects of Water Type

Before the start of the experiment, Munich well water (Mw) had a conductivity 197% higher than Volvic water (Vw), likely due to the higher contents in calcium and magnesium in Mw by 611% and 160%, respectively (Table S4). After a 4-week exposure, the two water types were quite similar in nutrient concentration and pH. The conductivity remained consistently higher in the Mw treatments during all 4 weeks, but the difference between the 2 water types was reduced from 440 µS cm^{-1} at the start of the experiment to 70 µS cm^{-1} after 4 weeks (Figure S1). Pirimicarb and tebuconazole declined but were still found at the end of the experiment (30–90%) and did not differ between Vw and Mw, but terbuthylazine was not found in any of the samples (Table S4). The decline of pesticides, including copper, did not differ between the two temperatures.

Overall, the water type did not strongly affect the stressor toxicity, nor did it significantly affect the growth of the different functional groups. The 95% ellipses for Mw and Vw overlapped in the PCAs, confirming this (Figure 2C). The water type, however, caused a small though significant increase in biomass of *E. nuttallii* in Mw compared with Vw (Figures S2 and S3, Table S5). This resulted in only a marginal effect on total macrophytes ($p = 0.07$). An interaction between the water type and ARO was also observed for periphyton (Figure 3). The water type had no effect on the other primary producers or consumers, nor did it modify the temperature or ARO toxicity towards them. Primary producer proportions were also highly comparable in both water types for the same treatment (Figure S4).

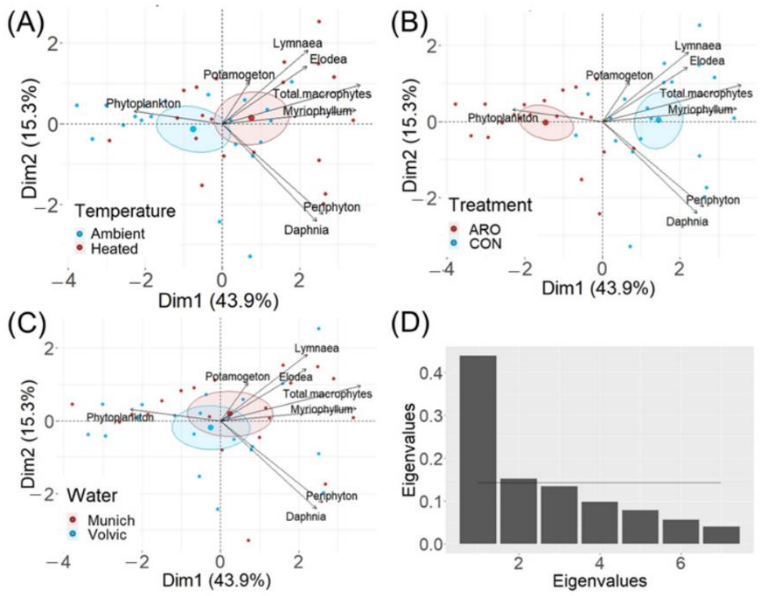

Figure 2. Microcosms. Principal components analysis plot showing the most significant axes and all response variables. The temperature, treatment and water effects are projected as supplementary data: (**A**) ambient (blue) or heated (red), (**B**) CON (blue) or ARO (red) and (**C**) Volvic (blue) or Munich well water (red). (**D**) Eigenvalue scores of the 7 main axes.

3.2. *Stressor Effects on the Primary Producers*

3.2.1. Microcosms

Both the macrophytes and periphyton respond negatively to the ARO, whereas phytoplankton responds positively (Figure 3). The total macrophyte dry aboveground biomass in the ARO treatments decreases by 60% compared with the control (Figure 3). Periphyton chl-*a* also decreased by 60% in the ARO treatment. The reduction in macrophyte and periphyton biomass was accompanied by an increase in phytoplankton biomass, which measured on average 1338% higher in the ARO treatments compared with the controls. The pesticide effects on phytoplankton were much stronger in the cold treatments, accounting for the overall strong pesticide effect (Figure 2). Warming on the other hand promoted total macrophyte biomass. The dominant but also most sensitive macrophyte species was *M. spicatum*, which significantly decreased in the ARO treatments by 65% compared with the control, and increased by 70% in the heated treatments compared with the ambient. The other two macrophyte species grew little and were not strongly affected by the stressors.

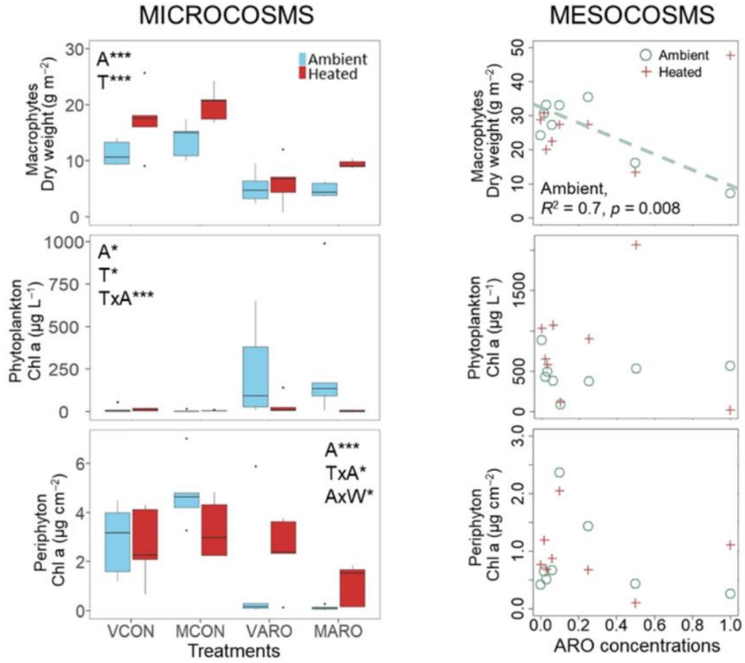

Figure 3. Stressor effects on the final aboveground biomass of all macrophytes, chl-*a* concentration of phytoplankton and periphyton in the microcosms (**left**) and mesocosms (**right**). Microcosms: VCON = Volvic control; MCON = Munich well water control. ARO; VARO = Volvic ARO; MARO = Munich well water ARO. Box plots of 5 replicates showing the median, 25 and 75% percentiles, lowest and highest whiskers (as Q1 − [1.5 × IQR] and Q3 + [1.5 × IQR], respectively), and outliers (dots). A = ARO, T = Temperature and W = Water. Mesocosms: Regression plots with the ARO concentration gradient along the *x*-axis. Mesocosms: R^2 and *p*-value from the linear regression. For the microcosm experiment, the significance levels are shown as * $p < 0.05$; *** $p < 0.001$.

3.2.2. Mesocosms

Not all the effects observed in the microcosms could be seen in the mesocosms. ARO effects on the primary producers were more comparable with the microcosms at ambient temperature. At ambient temperature, the total macrophyte biomass is negatively related to the ARO level with a decrease of 70% in the highest ARO compared to the control (Figure 3). However, the highest macrophyte biomass can be observed in the 1.0-H (ARO concentration in the heated mesocosms) treatment, showing no clear overall response pattern to the stressors (Figure 3). Both *M. spicatum* and *E. nuttallii* reach their maxima in the 1.0-H treatment and mimima in the 1.0-A treatment (Figure S3). *M. spicatum* produced the most biomass (181.4 g) among the macrophytes, followed by *E. nuttallii* (131.9 g) and *P. perfoliatus* (111.7 g). Similar to the microcosms, *M. spicatum* grew better in the heated microcosms compared with the ambient.

Phytoplankton reaches its highest biomass in the 0.5-H mesocosm and the lowest in the the 1.0-H concentration (Figure 3). Periphyton was not abundant in the mesocosms. The highest and lowest chl-*a* content were measured in the 0.1-A and the 0.5-H treatments, respectively (Figure 3).

Again, comparing only the ambient mesocosms, the 0.1 ARO treatment had the highest periphyton and the lowest phytoplankton biomass, similar to the microcosms. Additionally, although linear or quadratic models showed no correlation, the graphs point toward possible inverse relationships between phytoplankton and periphyton at intermediate ARO concentrations.

3.3. Stressor Effects on the Primary Consumers

3.3.1. Microcosms

The grazers were negatively affected by the pesticides. *L. stagnalis* lengths are on average 20% smaller in the ARO compared with the control (Figure 4). Warming, on the other hand, promoted their growth by 11%. A positive correlation was observed between *L. stagnalis* growth rate and *M. spicatum* biomass (Pearson, $r = 0.72$, $p < 0.0001$), but between *L. stagnalis* and the other macrophytes.

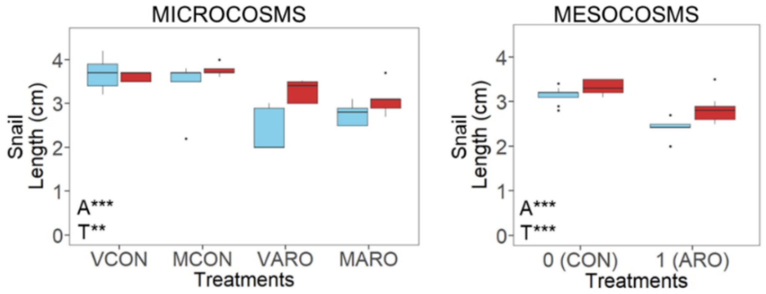

Figure 4. Stressor effects on the final length of snails in the microcosms (**left**) and mesocosms (**right**). Box plots of 5 replicates in the microcosms and 10 replicates in the mesocosms showing the median, 25 and 75% percentiles, lowest and highest whiskers (as Q1 − [1.5 × IQR] and Q3 + [1.5 × IQR], respectively), and outliers (dots). Microcosms: VCON = Volvic control; MCON = Munich well water control. ARO; VARO = Volvic ARO; MARO = Munich well water ARO. Mesocosms: 0 (CON) = control in Munich well water; 1 (ARO) = highest ARO concentration in Munich well water. ** $p < 0.01$; *** $p < 0.001$.

The highest *Daphnia* numbers are reached during week 2 with the controls showing 82% more individuals L^{-1} than the ARO (Figure 5). By the end of the 4-week exposure, the numbers in the controls reduced from 1213 ± 883 to 345 ± 329. *Daphnia* were controlling the phytoplankton top-down, over time in the controls. While ARO significantly suppressed *D. magna* development during all 4 weeks, it significantly promoted phytoplankton development from weeks 2 to 4. The total average biomass of *Daphnia* in the controls at the end of the experiment was 73% higher compared with the ARO. Temperature effects on plankton were measurable only in week 1, with an increase in both *Daphnia* numbers (Figure 5) and phytoplankton (Figure S5).

3.3.2. Mesocosms

Similar to the microcosms, the snails are affected negatively by ARO with a 16% reduced length in the 1.0 ARO compared with the control (Figure 4).

In contrast to the microcosms, *D. magna* was not part of our zooplankton community in the mesocosms. The natural zooplankton community comprises nine functional groups (Figure 5). The most prevalent morphotypes were calanoid copepods, *Daphnia longispina* and *Bosmina*, together representing over 80% of the community. During the experiment, the community structure shifts in all enclosures (Figure 5). The dominant groups at the end of the experiment were cyclopoid copepods, *Simocephalus* and *Chydorus*, together representing over 90% of the community. On average, the total zooplankton abundance rose from 8 individuals L^{-1} at the start to 130 individuals L^{-1} at the final sampling, but behaved very differently in each of the enclosures. The lowest density with 0.2 individuals L^{-1} was reached in the 1.0-A concentration. The highest density was reached at 711 individuals L^{-1} in the 0.25-A treatment. There was no clear correlation with ARO concentration or temperature.

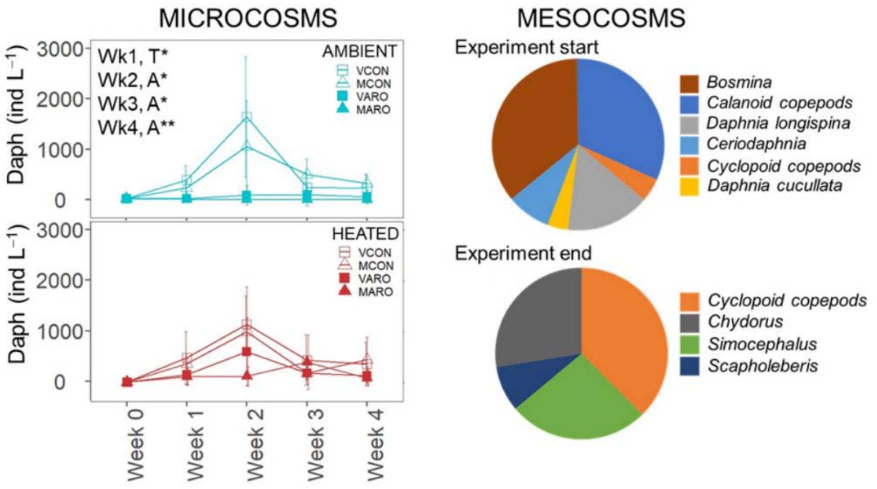

Figure 5. Impact of the stressors on the zooplankton in the microcosms (**left**) and mesocosms (**right**). In the microcosms, the temporal effects of the stressor on *Daphnia* numbers in the ambient (blue) and heated (red) microcosms are shown. Microcosms: VCON = Volvic control; MCON = Munich well water control. ARO; VARO = Volvic ARO; MARO = Munich well water ARO. Means ± SD, $n = 5$. A = ARO, T = Temperature and Wk = week. Mesocosms: zooplankton population at the start and end of experiment, pooled from all enclosures. Daph = *Daphnia*. * $p < 0.05$; ** $p < 0.01$.

In addition to zooplankton and snails added as experimental organisms, there were other invertebrates invading the mesocosms from early on. Eleven functional groups (based on families or genera) of insect larvae were distinguished, including Chironomidae, Aeschnidae, Libellulidae, Coenagrionidae, *Cleon*, *Dysticus*, Canidae, Culicidae, Hydrachnidia, Pleidae and Trichoptera (mostly *Hydropsyche*). Most of them belonged to four main groups: Ephemeroptera, Chironomidae, Odonata and *Chaoborus* (Figure 6). Among these groups, Odonata larvae had the highest average dry biomass (0.32 g m^{-2}), followed by Ephemeroptera and *Chaoborus*, each averaging 0.29 g m^{-2}. Chironomidae showed the least biomass (0.14 g m^{-2}) among these four groups. Odonata larvae were positively correlated with biomass of *M. spicatum* ($r = 0.5$, $p = 0.03$), but not with total macrophyte biomass ($r = 0.4$, $p = 0.1$). ARO did not affect the insect larvae. The average total insect larvae density stayed between 0.52 and 2.11 g m^{-2} in all the enclosures. The total insect larvae biomass was very similar between the ambient and heated enclosures. Ephemeroptera density, however, was halved by heating and chironomid density was even reduced by 70%. By contrast, the biomass of the predatory odonate larvae was increased by 700% due to heating.

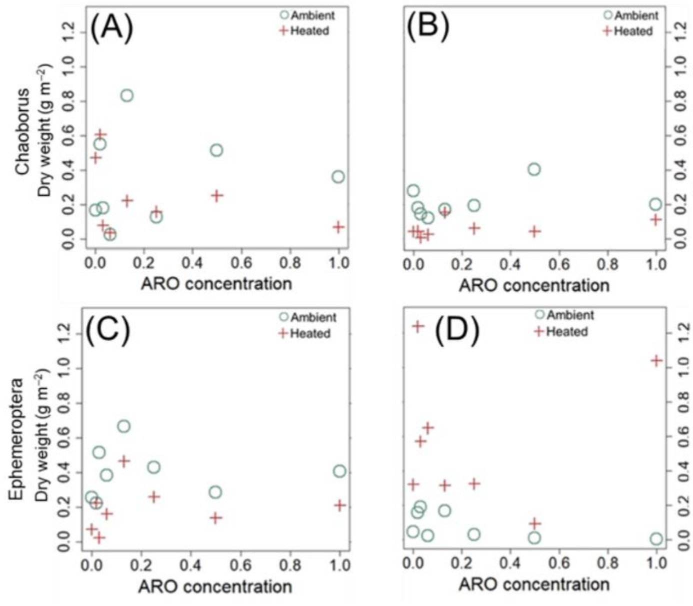

Figure 6. Mesocosms. Biomass of the four main groups of invasive invertebrates at the final sampling: (**A**) *Chaoborus*, (**B**) Chironomidae, (**C**) Ephemeroptera and (**D**) Odonata.

3.4. Regime Shifts in Micro- and Mesocosms

The microcosms and mesocosms were compared to determine whether similar patterns could be observed in the proportion of different primary producer groups. Despite not finding terbuthylazine in the microcosms and the rapid decline of pirimicarb in the mesocosms (Figure S6), among ambient treatments, the highest ARO of the mesocosms was the most phytoplankton dominated, similar to the ARO in the microcosms. In the microcosms, the control treatments were dominated by macrophytes (average proportion to standard stock organic carbon, macrophytes = 54%, average proportion of phytoplankton = 6.5%), while the ARO treatments had a comparable proportion of phytoplankton and macrophytes (average proportion of macrophytes = 31%, average proportion of phytoplankton = 35%). However, the apparent reduced resilience of the macrophyte-dominated state was significantly more pronounced in the cold ARO treatments (750% increase in phytoplankton proportion), compared with the warm ARO (171% increase in phytoplankton proportion) (Figure 7). As the mesocosms were more biologically complex, either macrophyte or phytoplankton dominance was observed among the enclosures. Intermediate ARO concentrations of the ambient mesocosms tended to be more macrophyte-dominated (Figure 7), while the control and highest ARO had comparable proportions of both primary producer groups. Trends of dominance in the heated mesocosms were less clear. The 1.0-H shows the strongest dominance of macrophytes, with 89% macrophytes and only 5% of both periphyton and phytoplankton, while the 1.0-A and the 0.5-H are the most phytoplankton-dominated accompanied by a low macrophyte biomass, both with ~70% phytoplankton and 25% macrophytes (Figure 7). The biovolume of macrophytes remains comparable between the micro- and mesocosms, but the zooplankton:phytoplankton ratio is relatively lower in the mesocosms (Figure S7).

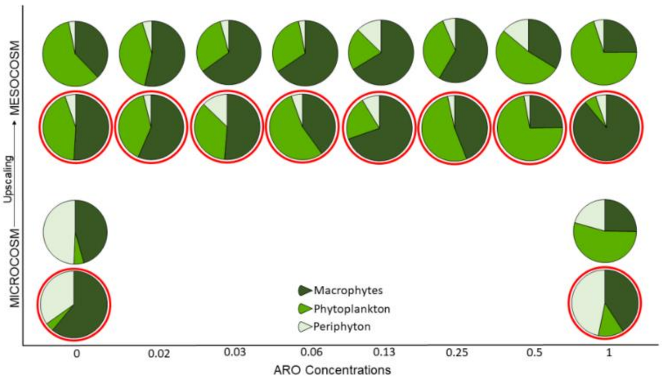

Figure 7. Microcosm–mesocosm comparison. Pie charts showing the relative proportion of primary producers in the microcosms (Munich well water treatments) and mesocosms when exposed to the ARO and the two temperatures. Heated treatments are represented by a red circle around the pie charts.

4. Discussion

Our experimental set-up and approach in upscaling from the highly controlled laboratory to the more complex outdoor conditions allowed us to confirm our first two hypotheses. First, while physiochemical properties of water have been show to affect aquatic organisms [9,10], adapting the water physiochemistry to suit the organism's needs can alleviate significant differences in effects due to water type. In our experiment, the nutrient levels in Mw were adjusted to more closely match the standard Vw, and, as a result, the water type did not affect the overall stressor effects and only showed some minor species-specific effects. Second, some stressor effects on the primary producers and snails could be replicated in the mesocosms. Our third hypothesis, however, was more complex. Some correlations between ARO and macrophytes could be found, and speculations on nutrient effects on the primary producers at intermediate ARO concentrations are possible. The lack of strong observable effects in the mesocosms shows that upscaling is challenging. However, an understanding of the role of enhanced species diversity and complexity, including more direct and indirect interaction effects and invasions, in modifying or even reversing strong responses observed in the microcosms, helps to evaluate how both experimental "scales" can be combined to increase the causal understanding and prediction of effects in real-world scenarios.

4.1. The Role of Water Type in Upscaling Experiments

Although water type had a species-specific effect on the macrophytes and modulated the impact of ARO to periphyton, the overall effects on the system did not differ between the two water types. The effect of the Munich well water (Mw) in promoting the growth of *E. nuttallii* may be related to the differences in the mineral status between the two waters, particularly the higher calcium and carbonate (higher alkalinity) concentrations in Mw compared to Vw, which can influence the photosynthesis or nutrient uptake capacity of this plant and thereby its growth [34,35]. *Elodea nuttallii* was much more efficient at phosphorus uptake in water that had higher calcium concentrations [34]. This effect on *E. nuttallii* only marginally affected the total macrophyte biomass, and thus did not significantly modify the overall response of the system. The interaction effect between ARO and the water type on periphyton growth may be due to compensatory feeding by snails linked to changes in the quality of the periphyton between Vw and Mw. Such compensatory feeding can make up for the low quality of some food sources, even if their growth is not affected [36]. Despite the conductivity remaining significantly higher in Mw treatments and the minor effects on *E. nuttallii* and periphyton, irrespective of water type, both controls were always dominated by macrophytes, both ARO treatments always dominated by phytoplankton, and a much stronger effect was observed in the cold microcosms. Similar phytoplankton blooms were observed in the experiments with single or pulsed ARO exposure [22]. Since ARO and temperature effects were the same in both water types and the water type itself did not significantly affect any of the main functional groups, that is, the macrophytes, phytoplankton, periphyton, *Daphnia* and snails, we were able to justify the use of the easily available Mw for our large outdoor mesocosms. Our results show that micro- and mesocosms can be performed using different water types and still allow comparability among research groups, provided the water type is chemically tested and adjusted for important physicochemical parameters that influence the studied biotic community.

4.2. The Role of Community Complexity in Upscaling

When we scaled up to the large outdoor mesocosms, some effects of the highest ARO on the primary producer groups at ambient temperature and on snails at both temperatures were similar to the microcosms. Although it is unclear whether terbuthylazine was present in the microcosms, this likely did not strongly affect the primary producers in both the micro- and mesocosms. Previous studies at similar concentrations have shown no negative effects of terbuthylazine on the primary producers (Polst et al., submitted; Vijayaraj, unpublished). In addition, other studies have shown similar algal blooms as in our microcosms when exposed to a combination of the same pesticides and nitrate (BPH, submitted, [22]), indicating that the resilience of the system is reduced when exposed to multiple stressors. In the mesocosms, at intermediate ARO concentrations, however, the positive effects of nitrate may have prevailed, thereby resulting in no observable negative effects by pesticides on the primary producer proportions. The effects on the primary producer proportion at the highest ARO of the ambient treatments in both experiments highlight a preservation of net negative effects in the mesocosms despite the increase in biological complexity. Warming, on the other hand, showed less clear effects and may not always threaten the macrophyte-dominated state. In fact, in combination with the pesticides, it may show either antagonistic or synergistic effects [37]. Pesticides have been shown to degrade faster at higher temperatures [38], but in both our micro- and mesocosms, pesticide decline did not differ between the two temperatures. The reduced effect of the ARO in the microcosm and the reversed effect in the mesocosm are most likely linked to increased or modified top-down control by the primary consumers in the heated treatments. The biotic structure and multiple stressor interactions can therefore complicate the prediction of warming effects on shallow lakes.

The lack of clearly observable net negative effects does not mean that no effects occurred. Our mesocosms had a rich biodiversity compared to the microcosms. Enhanced biodiversity offers a higher system stability [39] and potentially more direct and indirect biotic interactions, which probably masked clear direct stressor effects. This can potentially produce an outcome in a direction opposite of the direct effects. In the microcosms, direct or indirect stressor effects could be linked to individual species or interactions, as we included only the key organisms per trophic level. For example, the strong positive effect of the ARO on phytoplankton was possibly linked to reduced top-down control by *Daphnia*, which is also a key grazer in natural systems [40,41]. However, in the mesocosms, *Daphnia*, or large filter feeding cladocerans in general, were either absent or much fewer, and instead a complex zooplankton community existed. The observed changes in zooplankton assemblages may have influenced the strength of top-down and even bottom-up control [42,43]. One likely reason for the changes in the community is that the calanoids and large filter feeding cladocerans were more sensitive to the ARO and replaced by other more resistant groups. Our microcosm experiments show that *Daphnia* are strongly affected by the pesticides, which may explain their disappearance in the mesocosm experiments. Additionally, nutrient levels and the presence of alternative food sources may have also influenced the zooplankton assemblages. Du et al. [42] reported that at high nutrient concentrations, both an increase in chl-*a* and the presence of food sources other than phytoplankton, such as detritus and bacteria, promoted the biomass of cyclopoids and rotifers. This may be an explanation for why cladocerans and calanoids were eventually replaced by cyclopoids and other more competitive zooplankton species.

Phytoplankton community composition may in addition have been affected by selective grazing by zooplankton [44]. Such selective grazing may also allow certain zooplankton species to evade the effect of ARO on phytoplankton, with the non-selective feeders being more susceptible. Different sensitivities/responses of the zooplankton species to the ARO may also drive the phytoplankton response, for example, through changes in the zooplankton community size structure [45]. The complex community structure in the mesocosms also meant that the trophic cascade was more complex than in the microcosms. There was a high chance of omnivores feeding from more than a single trophic level (e.g., mixotrophic phytoplankton, or zooplankton, e.g., copepods, feeding on both algae and microzooplankton), thereby dampening potential strong direct top-down effects. Community structure may therefore play a defining role in modifying shallow lake responses to multiple pressures [46]. Allowing for a higher biodiversity in mesocosms is necessary to predict more closely real-world effects, especially in aquatic ecological risk studies with the aim of defining safe operating spaces. Such complexity allows us to follow the succession of species as well as determine the most sensitive and the fittest organisms under various stressor conditions. At the same time, controlled microcosm experiments that use key test organisms are fundamental in disentangling stressor effects on biotic interactions. Incorporating more natural communities in controlled laboratory conditions may provide a means of bridging this gap between the experimental scales.

4.3. The Role of Invasions in Upscaling

An additional trophic complexity was introduced through invasions in our mesocosms, which was not part of our microcosms. For many insects, freshly set-up mesocosms with dense macrophyte stands appeared an ideal opportunity for egg deposition. Soon, insect larvae belonging to many different functional groups appeared in all enclosures. Benthic chironomid larvae, for example, feed on detritus, algae and protozoans, as do Ephemeroptera larvae. *Chaoborus* and Odonate larvae are ambush predators feeding on zooplankton and insect larvae. By direct or indirect top-down control, these invading species likely affected planktonic primary producers and primary consumers, as well as benthic primary producers. The densities of these invading insects were comparable to natural densities [47]. They probably had serious consequences for our mesocosms, as they fed not only on zooplankton, thereby comprising a more advanced third trophic level compared to the copepods, but also on periphyton and may thus affect water quality and trophic state [48]. In the microcosms, the snails were the only key grazers of periphyton, and ARO effects on periphyton could be linked to the snails [22]. In the mesocosms, both the snails and the other invading invertebrates may have influenced periphyton biomass. Apart from the possible role of nitrate at low ARO concentrations, the lack of any strong effects on periphyton could be attributed to the compensation of snail feeding by that of invading insect larvae, which were less affected by ARO. Despite the invasions, however, both experiments point toward a general relationship between *M. spicatum* and consumers, with snails being influential in the microcosms through periphyton grazing [49] and odonate larvae in the mesocosms possibly by modifying top-down control of periphyton. In both cases, the primary consumers and/or odonate larvae developed better in the heated mesocosms and tended to buffer ARO effects. The very high biomass of odonate larvae in the highest ARO treatment of the heated mesocosm, which also happened to be the most macrophyte-dominated system, indicates the extent of the influence consumers may have in system response to stressors.

An Interesting implication is that because the ARO effects on snails remained the same at both scales, they should be considered a sensitive bio-indicator in freshwater ecosystems exposed to agricultural run-off. In fact, a normalised OECD test guideline for *Lymnaea* *stagnalis* reproduction has been adopted recently [50], showing their relevance as indicators of toxicant effects.

The stressors may also affect the primary producers differently, when embedded in branched or looped trophic chains with even or uneven trophic levels [24,51,52]. There was no top-down control on the snails, and the food web in the mesocosms was therefore branched rather than looped. The invasion by additional invertebrates resulted in a change from branched even–even food web as in the microcosms to an unintentional branched odd–even food web [24]. Since the top consumers are expected to control their prey or resources at an odd distance from themselves, any change in food web structure should have large consequences. The establishment of a strong carnivorous trophic level in our mesocosms, which can be influenced by resource availability [53] and feeding behaviour of the second trophic level [54], could therefore significantly have modified the direction of effects that we observed in the microcosms where only two trophic levels were included.

In warmer environments, poikilotherm organisms have a higher metabolic activity [55]. The foraging activities by *Chaoborus* and Odonata on other insects and zooplankton may therefore have increased, but the temperature may also have strongly regulated microplankton assemblages [42]. Therefore, the strong temperature effects observed on odonates and sometimes Ephemeroptera might have indirectly reversed the response of primary producers to the ARO, without observable interaction effects of temperature with ARO on the food web dynamics. Invasions by predators in large outdoor mesocosm experiments can therefore change net effects of stressors on primary producers and should also be well-monitored.

5. Conclusions

ARO lowered the resilience of alternative stable states in our model lake ecosystems, increasing the potential for regime shifts towards a phytoplankton-dominated state that supports fewer ecosystem services than macrophyte dominance [56]. These ARO effects were conserved at both scales at ambient environmental temperature, suggesting that ecosystems exposed to a combination of nitrate and pesticides are at risk for shifts to a degraded turbid state, and further action should be taken to reduce the use of these chemicals to protect shallow lakes. Increased temperature modified the effect of ARO, and the direction of this effect varied based on the ecological complexity of the system. At the microcosm scale, the negative ARO effect on macrophytes was still present at higher temperature but was buffered, while at the mesocosm scale, the highest temperature reversed the effect of the highest ARO treatment on primary producers. This modified effect may be due to changes in community structure, increased metabolic activity and strong top-down control by consumers at higher temperatures. Depending on the number of trophic levels and the feeding habits of the consumer community within the ecosystem studied, the temperature may modify the direction of ARO effects. Strong positive and negative effects observed in the controlled experiments can cancel out in complex outdoor studies to a zero net effect. No visible net ARO effects do not mean that there were no effects. While ARO effects may have been hidden within complexity in the mesocosms, our microcosms clearly indicated that ARO affects the biotic community. Controlled microcosm experiments are therefore important to disentangle the “effect pathways” of ARO within communities potentially important for real world scenarios. They may result in important hypotheses and a related design for testing when aiming to upscale the experimental system. Variable outdoor mesocosms allow us to identify gaps, and then improve the design and set-up of experiments at both scales to strengthen their complementarity. A comparison of the stressor effects at different scales and complexity is therefore a promising direction for risk evaluation studies in aquatic ecology and ecotoxicology.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w14040581/s1: Figure S1: Microcosms. Weekly measures of water physico-chemistry; Figure S2: Hedges' g effect sizes calculated from the F-statistic; Figure S3: Stressor effects on macrophytes in the micro- and mesocosms; Figure S4: Microcosms. Relative proportions of primary producers; Figure S5: Microcosms. Weekly measures of the absorbance of water at 663 nm; Figure S6: Mesocosms. Pesticide concentrations measured over 8 weeks; Figure S7: Biomass per volume of macrophytes and zooplankton:phytoplankton ratios in the micro- and mesocosms; Table S1: ARO concentrations and temperatures of micro- and mesocosms; Table S2: Starting size of biotic community in micro- and mesocosms; Table S3: Morphotypes used in the micro- and mesocosms; Table S4: Comparison of physico-chemistry between Volvic and Munich well water before and after exposure; and Table S5: Statistical details for individual and combined stressor effects on the different functional groups.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not yet publicly available, but will be deposited in local repositories (Université de Lorraine and Ludwig-Maximilians University), and are available upon request once the CLIMSHIFT project is finished.

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6. Discussion

Shallow aquatic systems within agricultural landscapes are often affected by surface run-off transporting nutrients and pesticides, so called agricultural run-off. In these systems, high nutrient concentrations can lead to regime shifts from a macrophyte-dominated clear water state to a phytoplankton-dominated turbid state, which thereby impairs the ecosystem services of shallow aquatic systems (Hilt et al., 2017). Such regime shifts can further be facilitated by elevated temperatures occurring during heatwaves or due to global warming. Assessing the effects of these multiple stressors on regime shifts is the aim of my thesis. Three main objectives were identified and targeted with my work:

1) Assessing the interactive effects of combined agricultural stressors on regime shifts between primary producers.
2) Analysing if the risk of regime shifts caused by multiple agricultural stressors is modified at higher temperatures.
3) Validating the results obtained in objectives 1 & 2 for different scenarios reflecting possible in-field scenarios. In the first part (a) the effects of exposure via the sediment are clarified, in the second part (b) the role of higher trophic levels is clarified.

Towards these objectives I will discuss in the following the interacting effects of nitrate and pesticides and how their combined exposure leads to regime shifts. I highlight the role of an adapting phytoplankton community (objective 1, chapter 7.1). Further, I discuss the role of elevated temperatures on regime shifts by comparing the effect of elevated temperatures individually and combined with ARO (objective 2, chapter 7.2). In the last part I discuss factors of relevance in the field that were included in the experimental settings, in particular the exposure pathways and fate of ARO, trophic complexity with a potential community adaptation, and temporal dynamics of stressor effects (objective 3, chapter 7.3). I conclude my thesis with an outlook to the recovery of ARO-induced regime shifts, further considerations of multiple stressors in risk assessment and measures needed to prevent regime shifts in the first place.

6.1 The effect of agricultural run-off (ARO) on the first trophic of shallow lakes level – phototrophic communities

High nitrate concentrations originating from agriculture are the driving factor behind phytoplankton growth and consequently the regime shifts reported in literature (Moss et al., 2013). Besides nitrate, pesticides are used extensively in agriculture and are major pollutants in aquatic systems. Agricultural run-off transports both, nitrate and pesticides, in nearby shallow aquatic systems where these co-occurring stressors can possibly interact. Since these two stressors have an opposing mechanisms – growth stimulating versus growth inhibiting – their co-occurrence may affect growth of phototrophic communities and ultimately the occurrence of regime shifts. To understand the effect of nitrate and pesticides on the shading potential of phytoplankton, I excluded, in a first experimental-driven approach, higher trophic levels in my experimental settings and focused on the biological interactions of the phototrophic communities. These are key players in the occurrence of regime shifts from macrophyte- to phytoplankton-dominated states. To further disentangle potential ecological mechanisms on the level of primary producers, a process-based modelling approach was used.

In the following, I summarize the results related to the first objective, and then discuss the role of stressor interactions and the adaptation potential of the phytoplankton community as an important mechanism responsible for regime shifts.

6.1.1 Summary of the key results related to objective 1: Synergistic interactions of multiple agricultural stressors lead to regime shifts between primary producers

The experimental approach applied in the *Stressor Interaction* chapter 3 revealed that nitrate-induced phytoplankton growth alone did not provide sufficient stress to limit macrophyte growth and thereby it did not induce a regime shift. These occurred only with co-occurring exposure of nitrate and pesticides, despite comparable levels of phytoplankton density in the treatments with individual nitrate and the combined treatment including pesticides. Repetition of the combined treatment in the experiments of the other chapters lead to comparable results (figure 18). Ultimately, synergistic interactions of nitrate and pesticide led to the observed regime shifts. Using the combined experimental and modelling approach in the *Process-based Modeling* chapter 4 revealed an adaptation of microalgae communities to the herbicide exposure.

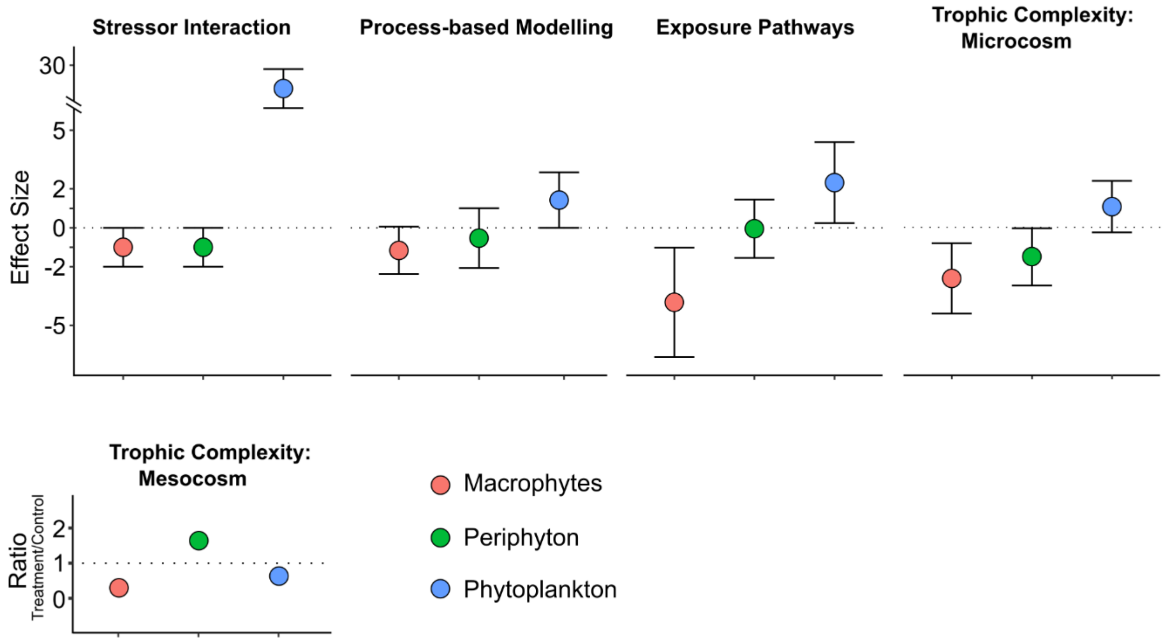

Figure 18 | Overview of the relative effects on the biomass of the three phototrophic communities of shallow aquatic systems caused by the ARO mixture tested across all experiments at ambient temperature (see table 2). Results from microcosm studies are shown as effect sizes ± standard error (*Stressor Interaction* chapter 3) or ± confidence interval (in the *Process-based Modelling*, *Exposure Pathways* and *Trophic Complexity* chapters 4, 5 & 6). Mesocosm experimental results from the *Trophic Upscaling* chapter 6 are shown as ratio in comparison to the control treatment as no effect sizes or variance could be calculated due to the lack of replicates. For details, e.g. which biological endpoint (e.g. chlorophyll a concentration or dry weight) were used for the respective calculations, see the individual chapters.

6.1.2 Interaction of stressors increase the risk of regime shifts

Opposing to the widespread understanding that high nitrate respectively nitrogen concentrations comparable to the ones used in my experiments increase phytoplankton growth, thereby shading of macrophytes and finally lead to regime shifts (Moss et al., 2013; Olsen et al., 2015), no nitrate-induced regime shifts were observed in the experimental settings of this thesis (*Stressor Interaction* chapter 3). A reason for this discrepancy maybe found in methodological-related effects e.g. too shallow microcosms that limit the realistic representation of real ecosystems. This way macrophytes could have still gained enough light as they were too close to the water surface to experience strong shading by phytoplankton. On the other side, regime shifts were found at similar phytoplankton levels when pesticides co-occurred (*Stressor Interaction* chapter). Contrasting to this finding, Gomes & Juneau (2017) and Brain et al. (2012) found that the toxicity of herbicides with the same mode-of-action as terbuthylazine decreases with decreasing light availability. Phototrophic organisms can produce more pigments to counteract the lower light availability caused by shading or as a response to low-dosed herbicides (Cedergreen et al., 2007; Gomes and Juneau, 2017). This mechanism can lead to an even higher photosynthetic efficiency and limit the effects of shading or herbicides (Hormesis effect, see Cedergreen et al., 2007). Nevertheless, following the subsidy-stress and stressor addition concepts, shading by phytoplankton and herbicides should both limit the growth of macrophytes once certain thresholds are exceeded when exposed in combination (figure 19). Both, the direct toxic stress exerted by herbicides and the indirect stress by nitrate-induced shading, act synergistically at high concentrations but interactions may differ along the gradient (*Stressor Interaction* chapter 3). A mesocosm experiment performed by Wendt-Rasch et al. (2004) indicates such addition respectively synergism as the toxic effect of herbicides is increased in turbid conditions and supports our findings.

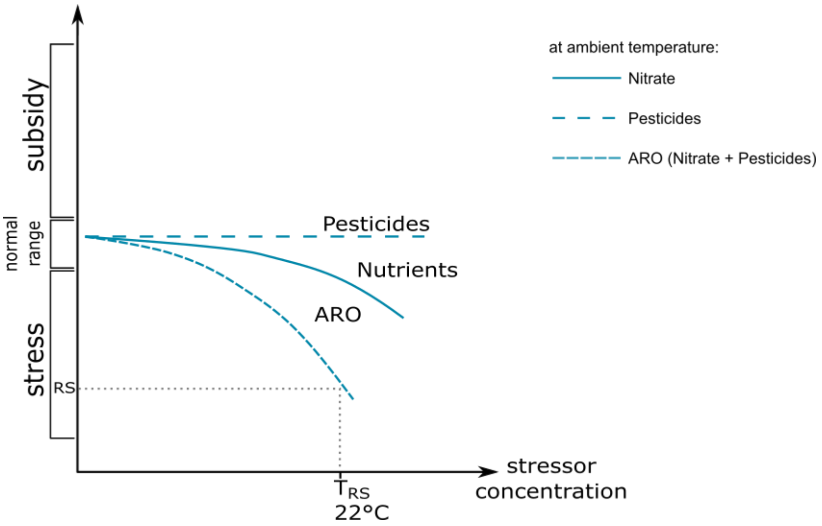

Figure 19 | The subsidy-stress concept (see also Fig. 4 Intro) now adjusted to the effects of the agricultural stressors from the perspective of the macrophyte-dominated clear water state according to their effects observed in the *Stressor Interaction* experiment. Only the combined treatment of nitrate and pesticides as ARO led to regime shifts (RS) at a certain threshold (T_{RS}).

Concluding, the limits at which macrophytes can counteract the negative effects by the direct toxic stress and the indirect stress by shading were exceeded with the ARO concentrations tested in this work. Synergistic interactions of co-occurring pesticides and nitrate-induced shading led to stronger effects on macrophytes, and clear regime shifts to phytoplankton dominated states. Furthermore, this result suggests that the role of pesticides on the occurrence of regime shifts is underestimated, and perhaps explains some of the remaining variability in studies that focus exclusively on nutrients (e.g. Beaulieu et al., 2013).

6.1.3 The high adaptation potential of the phytoplankton community is contributing to the increased risk of regime shifts

Long-term direct impact of pesticides on growth of phototrophic organisms or communities is difficult to analyse as they can adapt towards pesticide exposure over longer times via two means: 1) Physiological acclimation e.g. via increased pigment production (Gomes and Juneau, 2017), or 2) adaptation of the community through selection of tolerant species (Tlili et al., 2015). The range of herbicide concentrations in which macrophytes can acclimate to the exposure seems to be rather small. Cunningham et al. (1984) found recovery of *P. perfoliatus* at 0.31 µg L^{-1} atrazine but not at 1.2 µg L^{-1} atrazine despite fivefold increase in chlorophyll a content, which is comparable to the lower end of the environmental relevant herbicide concentrations tested in this work. Adaption of the macrophyte community via species selection does not take place within the timeframe at which regime shifts occur (<1 year). Microalgae communities on the other hand can adapt to exposure much faster due to their low generation time (Bérard and Benninghoff, 2001; Schmitt-Jansen and Altenburger, 2005). Thereby, microalgae communities can adapt to pesticide pollution and increase their tolerance within the timeframe of regime shifts, which is conceptualized in the concept of pollution-induced community tolerance (PICT, Tlili et al., 2015).

Testing species and community adaption via the PICT approach is challenging and a time intensive task, therefore such data in terms of regime shifts or even for multiple groups of phototrophs within a shared ecosystem are non-existent. In the *Process-based Modelling* chapter 4 I targeted this problem with a novel modelling approach and thereby have proven the adaption of microalgae communities. Concluding, phytoplankton communities adapt to ARO exposure, and this adaption contributes to regime shifts, thereby answering the second part of our first objective.

6.2 Effects of climate change on shallow aquatic systems

Higher average global temperatures as well as more frequent and intensive heatwaves are the apparently biggest effect of climate change on freshwater ecosystems (Dokulil et al., 2021; Woolway et al., 2021; Zhang et al., 2022). Lake water temperatures are predicted to increase by +4°C during heatwaves in low-greenhouse-gas-emission scenarios and even higher in scenarios with higher greenhouse gas emissions (Dokulil et al., 2021; Woolway et al., 2021). These increased temperatures are likely leading to regime shifts but can also interact with agricultural run-off and modify their potential to induce regime shifts (Paerl and Huisman, 2008; Moss et al., 2011). Whether the risk of ARO-induced regime shifts is modified at elevated temperatures is the second objective in this thesis. An increase in water temperature was therefore included in all experimental settings as a separate control treatment and in combination with the respective ARO treatments. This experimental approach revealed key results presented in the following paragraph and is discussed afterwards.

6.2.1 Summary of the results related to Objective 2: Elevated temperatures modulate the risk of ARO-induced regime shifts

Higher temperatures (ranging from +2.5°C to +4°C in the experimental settings) alone did not promote regime shifts and even increased macrophyte growth (see e.g. *Stressor Interaction* chapter). Nevertheless, higher temperatures affected ARO-related effects in each experiment as shown in the overview in table 3.

Table 3 | Overview of the effects of higher temperature on ARO-induced regime shifts.

Chapter	Effect of elevated temperature	Risk of regime shifts
Stressor Interaction	lowers thresholds for ARO-induced regime shifts	increased
Process-based Modelling	accelerates metabolism and decreases herbicide sensitivity of phytoplankton	increased
Exposure Pathways	negates negative effect of ARO exposure via sediment	unaffected
Trophic Complexity	varying effect along the ARO gradient; Reversed effect at the highest ARO concentration	too complex to conclude

In the *Stressor Interaction* chapter 3, higher temperatures decreased the thresholds for ARO-induced regime shifts. In the *Process-based Modeling* chapter 4, the model settings in which higher temperature did decrease the sensitivity to the herbicide, had the best fitting. In the *Exposure Pathways* chapter 5, higher temperature negated the negative effects of sediment-exposed ARO. In the *Trophic Complexity* chapter 6, higher temperature reversed the ARO-related effect at higher biological complexity at the highest ARO concentration in the mesocosm experiment. Overall, the reversed interaction of elevated temperature and ARO must be highlighted, as elevated temperatures alone did not induce regime shifts. When combined with ARO the risk of regime shifts can be increased as most clearly seen in the *Stressor Interaction* chapter 3 (figure 20).

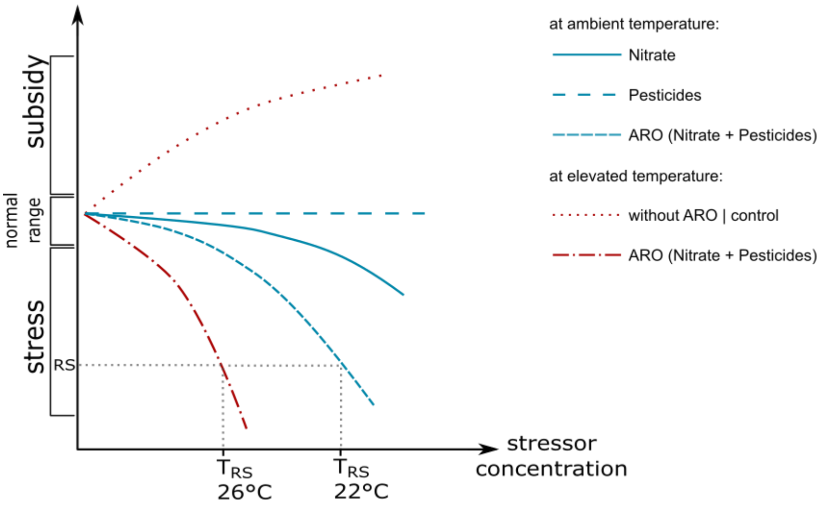

Figure 20 | The subsidy-stress concept (see also Fig. 6 Intro) now adjusted to the effects of the agricultural stressors and elevated temperature from the perspective of the macrophyte-dominated clear water state according to their effects observed in the *Stressor Interaction* chapter 3. While elevated temperature alone further subsidized the macrophyte-dominated state, it lowered the threshold for ARO-induced regime shifts (T_{RS}).

The effects of higher temperatures on ARO-induced regime shifts are more subtle and depend very much on additional factors as the range of ARO concentrations, the exposure pathway and complexity of the ecosystem. It seems that higher temperature rather modulates the effects of ARO in multiple directions depending on the experimental settings than a clear one-directional effect on ARO-induced regime shifts and hints to the need of a mechanistic understanding of the mechanisms behind stressor interactions.

6.2.2 The direct effect of elevated temperatures is unlikely to induce regime shifts

Many studies observed or predict increased phytoplankton growth in shallow lakes due to elevated temperatures (Paerl and Huisman, 2008; Kosten et al., 2012; Lürling et al., 2013; Richardson et al., 2019), while other studies predict macrophyte dominance (Mckee et al., 2002; Cao et al., 2015; Hansson et al., 2020). In an experimental mesocosm setting, Zhang et al. (2022) found a trend to periphyton dominance as a consequence of heatwaves. The results obtained in my work strengthen the argument of increased macrophyte growth in shallow aquatic systems at higher temperatures without agricultural pollution. Apart from the direct effects on growth of primary producers further temperature-related indirect effects may occur that can modify the risk of regime shifts. Higher remobilization of nutrients from the sediments could contribute to regime shifts (Duan and Kaushal, 2013) but was not found in the *Exposure Pathways* chapter 5. Further, higher temperatures can influence bottom-up control of filter feeders and grazers through changes in the food quality (macrophytes and microalgae) as well as top-down controlling feeding behaviour of snails (Moore et al., 1996; Zhang et al., 2019), but these were not explicitly tested in my experimental work. Ultimately, higher temperature without further agricultural pollution as studied in this thesis do not indicate an increased risk for regime shifts, neither by direct nor indirect effects. The opposite is the case, higher temperatures strengthen the macrophyte-dominated clear water state in shallow aquatic systems.

6.2.3 Elevated temperatures can interact with ARO to modify regime shifts

As shown in this thesis, effects of higher temperatures on ARO-induced regime shifts are too complex to be summarized in one clear statement. Further comparison to literature is difficult since only very few comparable studies exist. While remote and meta-studies show an increased risk of regime shifts when connecting land-use and higher temperatures (e.g. Beaulieu et al., 2013; Jeppesen et al., 2011; Kakouei et al., 2021), experimental studies are often limited in their conclusion regarding ARO-induced regime shifts. For example, Allen et al. (2021) found no modulation of ARO-related effects by elevated temperatures in a comparable experimental setting. Similarly, Zhang et al. (2022) found effects related to regime shifts when co-exposing nutrients and glyphosate at higher temperature, yet interactions of temperature and pollution were only found for one macrophyte species. In the *Trophic Complexity* chapter 6, ARO at elevated temperature had a positive effect on periphyton growth too and adds further arguments to the ongoing discussion on the role of periphyton in shallow lakes (e.g. Vadeboncoeur et al., 2021; Wijewardene et al., 2021) and if a periphyton-dominated clear water state is becoming a third alternative state due to climate change and agricultural pollution. Ultimately, elevated temperatures can interact with ARO, as highlighted in figure 20, and can thereby modify ARO-induced regime shifts. The overall effects of higher temperatures are subtle and depend on further environmental factors.

6.2.4 The effects of Climate Change go beyond elevated temperatures

The consequences of climate change for shallow aquatic systems are much more versatile and complex than just elevated temperatures. Physico-chemical changes (e.g. increasing CO_2 concentration, pH), changes in hydrological regime (water volume, desiccation) and changes in external energy influx (radiation) are possible further threads to ecosystems and can influence ARO-induced regime shifts in aquatic ecosystems (Bates et al., 2008). For example, higher radiation increases pesticide degradation, a lower water volume increases concentrations of chemical stressors, and CO_2 promotes growth of algae (Delpla et al., 2009; Li et al., 2016). Further, an earlier start of the growing season can favour macrophyte dominance (Rooney and Kalff, 2000), giving the macrophytes a head start in the competition versus microalgae. The whole bandwidth of climate change-related effects on shallow aquatic systems and potential impacts on regime shifts seems challenging. Focusing on increasing temperatures due to heatwaves and global warming in general is an already urgent problem and a good starting point to investigate climate change related effects on shallow aquatic systems.

6.3 ARO-induced regime shifts in scenarios closer to field situations

Micro- and mesocosm experiments are a popular tool to test the effect of global stressors on aquatic systems (Benton et al., 2007; Stewart et al., 2013). Yet the question if and how far results from those experimental settings can be transferred to aquatic systems in field scenarios is still a matter of discussion (Carpenter, 1996; Spivak et al., 2011; Simmons et al., 2021). One major limitation for the transfer of micro- and mesocosm experiments towards field scenarios is the inclusion of higher trophic levels (Carpenter, 1996; Queirós et al., 2015). Additionally, in the context of agriculturally influenced landscapes, another limiting factor was identified, namely the exposure pathway via surface run-off or subsurface flow respectively binding of pesticides to sediment particles (Ulrich et al., 2013; Qu et al., 2017; Niu et al., 2021). The exposure via the sediment was tested in this study with a controlled microcosm experiment at laboratory scale. To test the effects of higher trophic levels, two experiments were performed, one indoor microcosm experiment with selected key species of the second trophic level and one outdoor mesocosm experiment with further addition of natural planktonic communities. By design, the outdoor mesocosm experiment considered additional environmental factors as shown in table 4.

Table 4 | Factors potentially affecting the validation and transfer of results obtained under the first two objectives of this thesis to scenarios closer to the field.

Chapter	Factor affecting
Exposure Pathways	exposure via the sediment
Trophic Complexity: - Microcosm experiment - Mesocosm experiment	higher trophic levels: - key species - natural zooplankton communities
Trophic Complexity: Mesocosm experiment	natural sun light wind & precipitation daily temperature fluctuations

In the following I shortly summarize and discuss the results of the respective chapters related to objective 3, starting with the first part of the third objective related to the *Exposure Pathways* chapter 5, followed by the second part of the third objective related to the *Trophic Complexity* chapter 6 and its two experiments including higher trophic levels.

6.3.1 Summary of the key results related to objective 3a: Exposure via the sediment does not lead to regime shifts

I have hypothesized that exposure via the sediment affects only rooted macrophytes, while exposure via the water leads to a regime shift to phytoplankton dominance, as it was observed in the other *Stressor Interaction* chapter 3 and *Process-based Modeling* chapters 4 which focused on the application via the water phase. The experimental results confirm this hypothesis and show that the effects caused via different pathways are more complex. Nitrate and the pesticides, in particular the insecticide pirimicarb, partly translocated from the sediment to the water column based on their physico-chemical characteristics. Nevertheless, while we saw macrophytes negatively impacted by sediment exposure, the effects were the strongest when ARO was exposed via the water phase and phytoplankton shaded macrophytes. Based on the results in this chapter I conclude that exposure of ARO via the sediment has a negative impact, but the impact is worse when exposed via the water column.

6.3.2 The fate of pesticides influences ARO-induced regime shifts

The effects of ARO associated with the sediment depend on the fate of pesticides and nitrate. Binding of pesticides to soil particles depends on the physico-chemical properties of the respective chemicals, with $\log K_{OW}$ and $\mathrm{Log}K_{OC}$ as a major descriptor of their binding tendency and consequently their bioavailability (Knauer, 2016, *Exposure Pathways* chapter 5). Hydrophilic substances, e.g. pirimicarb ($\log K_{OW} < 3$), are more easily resolved into the water column, while hydrophobic substances, e.g. terbuthylazine and tebuconazole ($\log K_{OW} > 3$), will be absorbed to the sediment particles. In the end, such processes greatly contributed to the prevention of regime-shifts in our experimental sediment-exposed scenarios (*Exposure Pathways* chapter). In the field the translocation of pesticides from the sediment to the water phase can be accelerated by sediment dwelling organisms (Bundschuh et al., 2016), which were not included in in the *Exposure Pathways* chapter 5. Further experiments including multiple sediment dwelling organisms and various pesticides with different physico-chemical properties could enhance the realisms of such studies and allow for further extrapolation to the individual conditions in the field. The list of potential factors influencing the fate of pesticides goes even further. The stability and degradation of pesticides often depends on exposure to ultraviolet radiation (Burrows et al., 2002; Remucal, 2014; Lian et al., 2021). As observed in the *Trophic Complexity* chapter 6, pesticide concentrations – in particular the ones of pirimicarb – decreased drastically in the outdoor mesocosm due to direct photolysis when exposed to natural sun light. The rapid degradation of this insecticide was of high importance for the temporal stressor dynamics in the outdoor mesocosms, which is further discussed in paragraph 7.3.6.

6.3.3 Summary of the key results related to objective 3b: Higher trophic levels barely modify regime shifts induced by agricultural run-off

In the *Trophic Complexity* chapter 6 complex systems including higher trophic levels were used to test the effect of ARO exposed via the water column on nature-like systems. In the microcosm experiment regime-opposing effects were observed due to the activity of filter feeders. In the mesocosm experiment relative effects promoting periphyton growth on the cost of macrophytes and phytoplankton growth were observed, yet phytoplankton was the dominating group after eight weeks of ARO exposure. At higher temperatures periphyton showed the strongest relative increase, while macrophytes were dominating the mesocosm at the highest ARO treatment. Looking at the effects along the whole ARO gradient, tested, the effects show an intermediate state of macrophyte dominance at lower ARO concentrations. At higher temperatures the effects respectively dominating phototrophic groups varies along the gradient and allows for no clear interpretation respectively prediction.

6.3.4 The effects of combined agricultural stressors stay the same across trophic complexity

A better understanding of stressor mechanisms across different trophic scales is achieved by a combination of experiments focusing on single or few species and experiments focusing on complex communities at ecosystem levels (Puche et al., 2020). Once an understanding of the main mechanisms is obtained, in case of regime shifts it is the nitrate-induced shading by phytoplankton, the addition of higher trophic levels increases realism and possibly modify the occurrence of regime shifts. To gain further insight, a closer look at Vijayaraj et al. (2022b) is helpful, who tested the interactions of nitrate and pesticides in systems with a comparable approach as used in my *Trophic Complexity* chapter 6 (table 5): In their study, Vijayaraj et al. (2022b) used the same microcosm setup including grazers and filter feeders as in the *Trophic Complexity* chapter of this thesis to disentangle the effects of nitrate and pesticides in comparison with their mixed effect. No regime shifts were observed in the nitrate treatment due to the feeding pressure by filter feeders. While daphnia and snail abundance was affected by the pesticides, no regime shift was observed for the pesticide treatment. In the ARO treatment, where nitrate and pesticides were combined, the interaction of nutrients and pesticides lead to a regime shift.

Table 5 | Comparison of the potential of ARO-induced regime shifts in dependence of higher trophic levels. Effects are shown for the original ARO concentrations highlighted in table 2.

Experiment	Higher trophic levels	Stressor	Regime shift
Stressor Interaction	no	Nitrate	×
		Pesticides	×
		ARO	✓
Vijayaraj et al. (2022b)	✓	Nitrate	×
		Pesticides	×
		ARO	✓
Trophic Complexity			
- Microcosm	✓	ARO	✓
- Mesocosm	✓	ARO	✓

Conclusively, comparing the results from Vijayaraj et al (2022b) and my own experiments, comparable interactions of nitrate and pesticides in the ARO mixture were found even with inclusion of higher trophic levels. The observed effect of ARO is comparable to the ones observed in the microcosm study focusing on phototrophs only in the *Stressor Interactions* chapter, and the microcosm study including filter feeders in the *Trophic Complexity* chapter. In the end, the conclusion drawn for the effect of ARO when tested on phototrophic communities holds true for systems with higher trophic complexity: Combined agricultural stressors induces regime-shift and is this effect is detectable even across experiments differing in trophic complexity.

6.3.5 Changes in the zooplankton community enable regime shifts

Despite the addition of representative key species, these indoor microcosm experiments were still simplified compared to complex communities in natural systems. Through the addition of a natural zooplankton community and openness to invasive species, the outdoor mesocosm in the *Trophic Complexity* chapter 6 not only had a second trophic level, but a more advanced third trophic level with species, e.g. Chaoborus and Odonata larvae, feeding on zooplankton. These omnivorous species likely limited the top-down control exerted by planktivorous zooplankton and thereby indirectly led to phytoplankton dominance. Such a top-down control leading to a phytoplankton bloom was also observed, for example, by Walsh et al. (2016) with the invasive zooplankton species *Bythotrephes* in Lake Mendodta (USA). This shows the limitations of laboratory experiments focusing on few selected key species in comparison to complex ecosystems as tested in the mesocosms (*Trophic Complexity* chapter 6) and found in the field. Further, this highlights that there are not only bottom-up mechanisms, e.g. nitrate induced phytoplankton growth, but also top-down mechanisms contributing to regime shifts when complex higher trophic levels are present.

6.3.6 Temporal dynamics of combined stressors after pulsed exposure

While answering the research questions of this thesis, an additional stressor-related factor gained my attention. The temporal dynamics of stressors influenced the experimental results and their transfer to field situations. Co-occurring stressors usually do not only differ in their intensity but also must overlap in time and space to be able to co-act on biological systems (Jackson et al., 2021; Polazzo et al., 2021). The agricultural stressors in this work were applied as a single combined pulse, but still differed in their temporal effect dimension. Nitrate concentrations in the water rapidly decreased through uptake by phytoplankton which rapidly transforms this inorganic stressor into an indirect biotic stress by limiting light availability for benthic phototrophs. Thus, the stressor nitrate had the strongest effects when phytoplankton peaked, inducing a unimodal distribution of the secondary indirect stressor (shading) over time. In contrast, pesticide concentrations decreased at a much slower rate than nitrate, but pesticides were not transformed into a secondary stressor (figure 21). Heavy metals as copper do not degrade over time as organic pesticides but can be absorbed by organic matter and can form compounds with hydrogen that reduces the toxicity of copper.

As shown in the *Exposure Pathways* chapter 5 and the *Trophic Complexity* chapter 6, pesticides show different degradation ratios based on exposure pathways and exposure to artificial or natural light. Despite synchronous exposure, the duration of exposure can differ for the different trophic scales as seen in the mesocosm experiment of the *Trophic Complexity* chapter 6, where zooplankton experienced only a short exposure period by the insecticide, while phototrophs were exposed to the herbicide for the whole duration of the experiment. Further, time-lagged effects are of importance and effects of stressors can still be found after the applied chemical has disappeared. Time-lagged effects were seen in the *Stressor Interaction* chapter 3, where the nitrate-induced phytoplankton bloom was limited in duration but still led to detectable long-lasting, respectively delayed effects of shading in macrophytes.

In the opposite and despite our comparable experimental setups, primary producer growth in the *Process-based Modeling* chapter 4 was lower and relative high nitrate concentrations were still found at the end of the experiment. Subpar growth of microalgae before inoculation and the original sampling of macrophytes used for the experiment from nearby aquatic systems late in the season may explain these discrepancies and indicate the importance of starting conditions in such laboratory experiments.

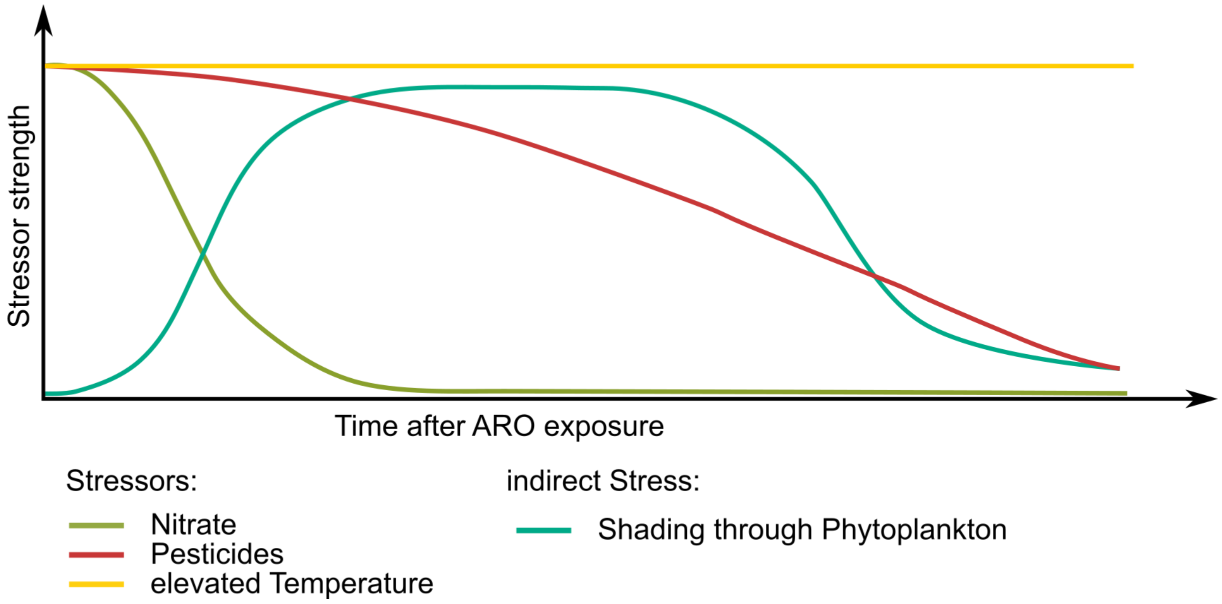

Figure 21 | Conceptual stressor strength over time as applied in this thesis. While the nitrate concentrations are rapidly transformed into phytoplankton and thereby to shading of macrophytes, pesticides degrade at slower rate and the elevated temperature stays constant throughout the observed time.

Another aspect raised by Jackson et al. (2021) is the exposure over several generation times. While regime shifts related to agricultural run-off occur within one generation of macrophytes, microalgae pass several generations within this time frame. This allows the microalgae community to adapt via selection of more tolerant respectively adapted species. Zooplankton species can adapt to stressor exposure via rapid micro-evolutionary processes within the span of a few generations and have an advantage to stressor exposure over several generations of no exposure (Jansen et al., 2011; Orr et al., 2021). Thus, adaptation to one or more stressors depends on temporal stressor dynamics, can occur within different trophic levels in parallel and contribute to synergistic interactions of multiple stressors with ecosystem wide effects.

7. Conclusion

The research performed in my thesis touches a broad range of topics, from stressor-interactions and alterative-stable states of ecosystem to community adaptation and the relevance of temporal dynamics of stressors. The discussed topics contribute to an enhanced understanding of regime shifts induced by agricultural run-off in the context of climate change. In the following I discuss implications of my results for the recovery from an ARO-induced regime shift, further needs for environmental risk assessment to derive ecological thresholds and implementation of measures related to the stressors highlighted in my thesis.

7.1 Recovery of the system to a macrophyte dominated state

The alternative stable state concept (Scheffer et al., 1993) explains that the first regime-shift to a phytoplankton-dominated clear water state happens at a higher nutrient concentration than the recovery-shift to a macrophyte dominated state, a so-called hysteresis effect (e.g. highlighted by Ibelings et al., 2007). In this thesis I have shown that pesticides increase the risk and lower the nitrate threshold concentrations inducing this first regime-shift. While the recovery of the system was not part of the work in this thesis, I can give a brief hypothetical outlook to the implications of ARO on recovery of shallow aquatic systems.

The recovery towards macrophyte dominance mostly depends on a few key filter-feeders, especially daphnia and mussels (Ibelings et al., 2007; Gulati et al., 2008). Representatives for both groups were found to be negatively affected by the ARO mixture. Invertebrates were affected by the insecticide and snails were affected by the fungicide, too. Yet these negative effects could be limited by the high photodegradation of the insecticide. Thus there is a chance that filter feeding communities recover quickly from the non-lethal ARO-effects and support the recovery of the system, while, at the same time, macrophytes still experience exposure to more persistent herbicides. As a consequence, recovery of macrophytes may takes longer than the re-establishment of a clear-water state. In both cases, higher temperatures can support the recovery due to the positive effects on filter feeders and macrophyte growth, unless critical temperatures are exceeded. Additional factors, e.g. close contact of mussels to contaminated sediment, additional trophic levels, or recycling of nutrients from detritus could further influence the recovery.

7.2 Ecological thresholds for risk management

The aim of risk assessment is to define acute and chronic thresholds based on adverse ecological effects for the protection of ecosystems. This approach is used for example in the water framework directive in which single species tests, micro- and mesocosm experiments as well as effect data from the field are used to derive threshold values for protection and establishment of a good state of ecosystems (SCHEER, 2018). While such thresholds exist for nitrate and pesticides, their combined effect when co-occurring is neglected. The work in this thesis offers an approach based on stable states of ecosystems covering the effects of multiple stressors in the whole ecosystem. The importance of synergistic effects deriving from interactions of direct and indirect stressor effects is highlighted and illustrates the complexity of stressor interactions in whole ecosystems. This finding is of importance for the diagnosis and prioritisation of multiple stressors. Further, this thesis provides a first look at the relevance of the abiotic stressor of elevated temperatures and heat waves on combined effects with other stressors, which is not yet implemented for risk assessment of chemicals in freshwater systems or their management (SCHEER, 2018). Building up on the foundation laid with my work, precise quantitative data on the combined effects of agricultural stressors on regime shifts in shallow aquatic systems are needed at best using crossed gradient studies with a changing ratio in the stressor mixture are needed (figure 22).

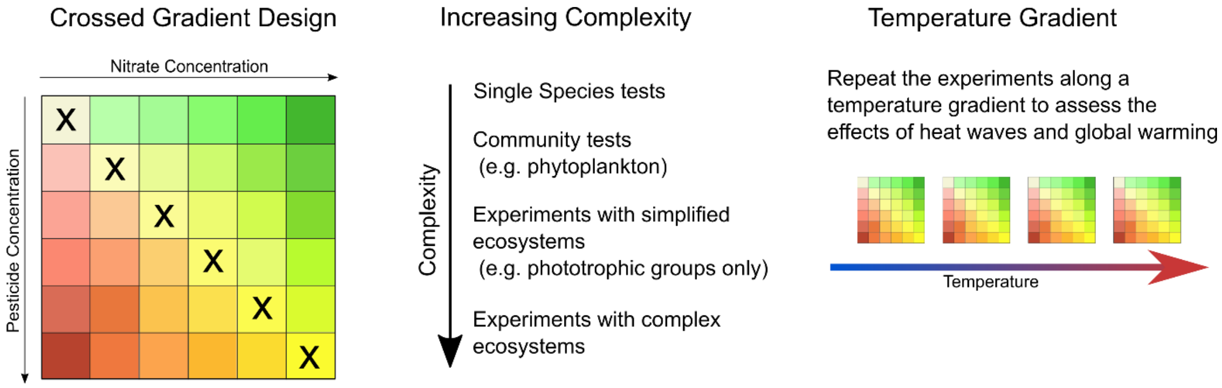

Figure 22 | Concept of experimental designs needed to clearly define thresholds for co-occurring nitrate and pesticides under global warming. The “X” in the left part of the figure marks the mixture rates used in my experiments.

In my work, the stressors were tested at a fixed mixture ratio but at differing concentrations (Figure 22, marked by “X”). Experiments at different scales of complexity and further consideration of increasing temperatures combined with modelling approaches would allow for derivation of future-prove thresholds respectively environmental quality standards values for protection of aquatic systems impacted by multiple agricultural stressors.

7.3 Implementation of ecological thresholds into management of aquatic systems

Ecological thresholds derived from risk assessment find their way into ecosystem management via implementation into legislation. For such an implementation, management thresholds are derived by applying a safety factor to these ecological thresholds, based on the quality of the available data. These management thresholds, often referred to as environmental quality standards, are the basis of which further mitigation measures are decided. But unlike larger aquatic systems, small and shallow aquatic systems within an agricultural landscape are often not included in legislative regulations like e.g. the water framework directive (Weisner et al., 2022). For these lager systems, riparian buffer strips are a well-established measure to limit the influx of agricultural run-off (Arora et al., 2010). Riparian buffer strips could not only limit the influx of agricultural run-off but also provide shade and thereby decrease water temperatures (Cole et al., 2020). In shallow ponds, lakes and slow-flowing streams in agricultural landscapes mitigation measures are rarely applied due to the lack of legal obligation. Including these aquatic systems in legislations aiming to protect aquatic ecosystems, e.g. the water framework directive or the EU nitrate directive, would enable regular monitoring of influx of agricultural run-off and force the use of such mitigation measures. This could ultimately prevent regime shifts and ensure preservation of the preferred clear water state and its manifold ecosystem services.

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Danke euch allen!

Declaration of Authorship

I hereby affirm that I have composed this dissertation by myself and only with the use of resources, data, and literature cited in the thesis. Those who provided assistance for the experiments, data analysis, or writing of the manuscripts are listed as co-authors or are mentioned in the acknowledgements of the respective publications.

Furthermore, I confirm that I have read and fully understood the regulations for a doctoral degree (“Promotionsordnung”) of the faculty of biology of the LMU Munich.

I did not obtain any assistance from a consultant for doctorate theses, or any third parties. I have not received any indirect od direct financial rewards in relation with the contents of this dissertation. This dissertation or parts of it have not been previously submitted as thesis or dissertation to the Ludwig Maximilians University of Munich or any other university.

Würzburg, 12. Dezember 2022

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Bastian H. Polst

Contribution to the individual publications

Chapter 3 – Stressor Interaction (Polst et al. 2022b, published in *Science of the Total Environment*): "Warming lowers critical thresholds for multiple stressor–induced shifts between aquatic primary producers."

The study design, setting up the microcosm and conduction the microcosm experiment and data analysis was mostly done by myself. The writing of the manuscript was done under my lead with the main part of the work done by myself:

Study design	80 %
Experimental work	95 %
Data analysis	95 %
Manuscript writing	70 %

Chapter 4 – Process based modelling (López Moreira M. et al., 2022, submitted to *Frontiers in Plant Science*): "Microcosm experiments combined with process-based modelling reveal differential response and adaptation of aquatic primary producers to warming and agricultural run-off."

The experimental part of the study was mostly done by myself with help during the final sampling from Sabine Hilt and other colleagues at the IGB Berlin. The model was developed by Gregorio A. López Moreira M. in collaboration with me, Sabine Hilt and Franz Hölker. Gregorio A. López Moreira M. and I contributed equally to the final manuscript. Further, co-authors contributed to the discussion and editing of the manuscript:

Study design	40 %
Experimental work	80 %
Data analysis	20 %
Manuscript writing	50 %

I hereby confirm the shared first authorship and equal contribution for this publication.

Chapter 5 – Exposure pathways (Polst et al. 2022a, submitted & in review in the *Journal of Applied Ecology*): “Exposure pathways matter: Aquatic phototrophic communities respond differently to agricultural run-off released via sediment or water.”

The study was designed with contributions from other Climshift members. The experimental part was mostly done by myself. Joey Allen contributed the microbial part during sampling and manuscript writing. Otherwise, data analysis and manuscript writing were primarily done by myself and co-authors contributed under my lead:

Study design	70 %
Experimental work	80 %
Data analysis	80 %
Manuscript writing	70 %

Chapter 6 – Trophic Complexity (Vijayaraj et al. 2022, published in *Water*): “Evaluating Multiple Stressor Effects on Benthic–Pelagic Freshwater Communities in Systems of Different Complexities: Challenges in Upscaling”

I contributed to the study by analysing the pesticides for both experiments. Further, I contributed to the mesocosm experiment by planning and setting up the mesocosm, applying the ARO mixture, monitoring of pesticides, sampling of biological parameters throughout the experiment and during the final sampling. I contributed to the results and to the discussion of the manuscript:

Study design	20 %
Experimental work	30 %
Data analysis	20 %
Manuscript writing	10 %

The contributions to the individual publications is confirmed by Prof. Dr. Herwig Stibor.

Publications

Polst B.H., Hilt S., Stibor H., Hölker F., Allen J., Vijayaraj V., Kipferler N., Leflaive J., Gross E.M., Schmitt-Jansen M. (2022): Warming lowers critical thresholds for multiple stressor-induced shifts between aquatic primary producers; Science of the Total Environment.

Polst B.H., Allen J., Hilt S., Stibor H., Gross E.M., Schmitt-Jansen M. (2022): Do exposure pathways matter? Aquatic communities respond differently to agricultural run-off released via sediment or the water phase; Journal of Applied Ecology, *in review*

Polst B.H., Moreira M.G.A.L., Leflaive J., Stibor H., Gross E.M., Schmitt-Jansen M., Hölker F., Hilt S. (2022): Microcosm experiments combined with process-based modelling reveal differential response and adaption of aquatic primary producers to warming and agricultural run-off.; *in preparation*

Vijayaraj V., Kipferler N., Stibor H., Allen J., Hölker F., Laviale M., Leflaive J., Moreira M.G.A.L., **Polst B.H.**, Schmitt-Jansen M., Hilt S., Gross E.M. (2022): Evaluating multiple stressor effects on benthic–pelagic freshwater communities in systems of different complexity: challenges in upscaling. Water.

Kipferler N., Vijayaraj V., Moreira M. G.A.L., Hilt S., Hölker F., Leflaive J., **Polst B.H.**, Schmitt-Jansen M., Laviale M., Stockenreiter M., Gross E.M., Stibor H. (2022): Design of a suspended benthic–pelagic shallow lake mesocosm system for multiple stressor and global warming research. L&O Methods; *in revision*

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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 Research.

Polst B.H., Anlanger C., Risse-Buhl U., Larras F., Hein T., Weitere M., Schmitt-Jansen M., (2018): Hydrodynamics alter the tolerance of autotrophic biofilm communities towards herbicides. Frontiers in Microbiology.

Conference contributions

Talks

Polst B.H., Hilt S., Moreira M. G.A.L., Hölker F., Stibor H., Leflaive J., Gross E.M., Schmitt-Jansen M.: Regime shifts in shallow aquatic ecosystems induced by multiple agricultural stressors in the context of climate change; Jahrestagung der Deutschen Gesellschaft für Limnologie e.V. 2021, Leipzig

Polst B.H., Allen J., Gross E.M., Hilt S., Stibor H., Leflaive J., Schmitt-Jansen M.: Exposure pathways matter – a microcosm study to evidence effects on phototrophic communities by agricultural run-off introduced via the sediment or the water phase; Shallow Lakes Conference 2021, virtual conference

Polst B.H., Gross E.M., Leflaive J., Hilt S., Stibor H., Schmitt-Jansen M.: Stressor interactions: regime shifts under multiple agricultural stressors; Symposium for European Freshwater Sciences 2021, virtual conference

Polst B.H., Gross E.M., Schmitt-Jansen M.: To shift or not to shift: Agricultural run-off induced regime shifts in phototrophic communities; Ecotoxiomics - 2nd International Conference on Microbial Ecotoxicology 2020, virtual conference

Polst B.H., Hilt S., Leflaive J., Stibor H., Schmitt-Jansen M., Gross E.M.: Agricultural run-off-induced regime shift in aquatic phototrophic communities changes with elevated temperature; Symposium for European Freshwater Sciences 2019, Zagreb

Polst B.H., Anlanger C., Koca K., Lorke A., Risse-Buhl U., Weitere M., Schmitt-Jansen M.: Herbicide sensitivity of stream biofilms grown at contrasting hydrodynamic conditions - a mesocosm study; Jahrestagung der Deutschen Gesellschaft für Limnologie e.V. 2017, Cottbus

Poster

Polst B.H., Lips S., Kipferler N., Vijayaraj V., Stibor H., Gross E.M., Hilt S., Schmitt-Jansen M.: Phototrophic communities of shallow lakes gain tolerance towards herbicides after exposure to agricultural run-off; Jahrestagung der Deuschen Gesellschaft für Limnologie e.V. 2022, Konstanz

Polst B.H., Vijayaraj V., Kipferler N., Allen J., Lopez G., Leflaive J., Hölker F., Hilt S., Stibor H., Gross E.M., Schmitt-Jansen M.: Warming lowers ciritical thresholds for multiple stressors induced regime shifts between primary produvers; SETAC Europe 2022, Copenhagen

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