

Supplementary Materials

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

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Table S1. ARO concentrations and temperature of micro- and mesocosms.

| ARO | Microcosms | Mesocosms |
|---|------------|---|
| Terbutylazine ($\mu\text{g L}^{-1}$) | 0, 3 | Gradient: 0, 0.02, 0.03, 0.06, 0.13, 0.75, 1.5, 3 |
| Pirimicarb ($\mu\text{g L}^{-1}$) | 0, 15 | Gradient: 0, 0.2, 0.5, 0.9, 1.9, 3.75, 7.5, 15 |
| Tebuconazole ($\mu\text{g L}^{-1}$) | 0, 90 | Gradient: 0, 1.4, 2.8, 5.6, 11.3, 22.5, 45, 90 |
| Copper (Cu, $\mu\text{g L}^{-1}$) | 0, 42 | Gradient: 0, 0.7, 1.3, 2.6, 5.25, 10.5, 21, 42 |
| Nitrate (N-NO ₃ , mg L ⁻¹) | 0, 9 | Gradient: 0, 0.1, 0.3, 0.6, 1.1, 2.25, 4.5, 9 |

Table S2. Starting size of biotic community in micro- and mesocosms.

| Species | Microcosm | Mesocosm |
|------------------------------|---|---|
| | Starting size, Average \pm SD | |
| <i>M. spicatum</i> | 10 cm (2 shoots) | 10 cm (10 shoots) |
| <i>P. perfoliatus</i> | 10 cm (2 shoots) | 10 cm (10 shoots) |
| <i>E. nuttallii</i> | 10 cm (2 shoots) | 10 cm (15 shoots) |
| Phytoplankton | $7.5 \times 10^5 \mu\text{m}^3 \text{mL}^{-1}$ | - |
| Periphyton | $8.35 \times 10^5 \mu\text{m}^3 \text{mL}^{-1}$ | - |
| <i>L. stagnalis</i> | 18.9 \pm 2.4 mm (1 individual per microcosm) | 10.7 \pm 3.7 mm (10 individuals per mesocosm) |
| <i>D. polymorpha</i> | 9.1 \pm 1.3 mm (3 individuals per microcosm) | 19.7 \pm 1.9 mm (10 individuals per mesocosm) |
| <i>D. magna</i> /zooplankton | 1391 \pm 17 mm (2.5 individuals L ⁻¹) | - (8 individuals L ⁻¹) |

Biovolume was not measured for the mesocosms as natural benthic and pelagic communities were mixed, but may have been lower in the mesocosms due to a longer incubation within the enclosures.

Table S3. Morphotypes used in the micro- and mesocosms.

| Species richness | | | |
|-------------------|------------------------|------------------|--------------------|
| Community | | Initial inoculum | Final sample |
| Microcosms | | | |
| Macrophytes | Chosen species | 3 species | 3 species |
| Phytoplankton | Chosen culture strains | 3 strains | Mostly green algae |
| Periphyton | Chosen culture strains | 5 strains | Mostly diatoms |
| Zooplankton | Chosen culture strains | 1 species | 1 species |
| Mussels | Chosen species | 1 species | 1 species |
| Snails | Chosen species | 1 species | 1 species |
| Mesocosms | | | |
| Macrophytes | Chosen species | 3 species | 3 species |
| Phytoplankton | Natural community | 30-50 species | 9 species |
| Periphyton | Natural community | | |
| Zooplankton | Natural community | 9 species | 5 species |

| | | | |
|-------------------------------|----------------|-----------|-----------|
| Mussels | Chosen species | 1 species | 0 species |
| Snails | Chosen species | 1 species | 2 species |
| Insect larvae (non-predatory) | Invasive | 0 | 5 species |
| Insect larvae (predatory) | Invasive | 0 | 6 species |

Table S4. Comparison of physicochemistry between Volvic and Munich well water before and after exposure.

| Before exposure | Volvic water | Munich well water |
|---|--------------|-------------------|
| Cond ($\mu\text{S cm}^{-1}$) | 223 | 663 |
| pH | 7.8 | 8.2 |
| Alk. (mM) | 0.64 | 2.9 |
| Cl (mg L^{-1}) | 14.9 | 14.7 |
| SO ₄ (mg L^{-1}) | 9.08 | 10.7 |
| Ca (mg L^{-1}) | 12.8 | 91.1 |
| Mg (mg L^{-1}) | 8 | 20.8 |
| Na (mg L^{-1}) | 11.7 | 8.9 |
| K (mg L^{-1}) | 6.1 | 1.5 |
| N-NO ₃ (mg L^{-1}) | 1.7 | 2.96 |
| N-NO ₂ (mg L^{-1}) | 0.08±0.04 | 0.07±0.01 |
| N-NH ₄ (mg L^{-1}) | 0.002±0.001 | 0.005±0.005 |
| DIN ($\mu\text{mol L}^{-1}$) | 210.7±20.8 | 310.6±26.1 |
| P-PO ₄ (mg L^{-1}) | 0.07±0.06 | 0.12±0.1* |
| P-PO ₄ ($\mu\text{mol L}^{-1}$) | 2.4±2.01 | 3.73±3.12 |
| NP molar ratio | 269.8±291.4 | 332.3±381.7 |
| 1h after exposure (ARO treatments) | | |
| Terbutylazine | - | - |
| Pirimicarb | 15.51±0.63 | 15.91±0.48 |
| Tebuconazole | 79±13.25 | 65±7.7 |
| Copper | 42.9±5.9 | 42.3±3.0 |
| After 4-week exposure (control treatments) | | |
| Cond ($\mu\text{S cm}^{-1}$) | 354±14.09 | 424±18.8 |
| pH | 8.0±0.24 | 8.16±0.05 |
| Cl (mg L^{-1}) | 21.49±0.62 | 21.30±0.48 |
| SO ₄ (mg L^{-1}) | 14.5±1.6 | 15.8±2.0 |
| N-NO ₃ (mg L^{-1}) | 0.16±0.10 | 0.2±0.1 |
| N-NO ₂ (mg L^{-1}) | 0.01±0.01 | 0.01±0.01 |
| N-NH ₄ (mg L^{-1}) | 0.09±0.12 | 0.08±0.09 |
| DIN ($\mu\text{mol L}^{-1}$) | 19.13±15.72 | 21.30±15.07 |
| P-PO ₄ (mg L^{-1}) | 0.07±0.03 | 0.05±0.03 |
| P-PO ₄ ($\mu\text{mol L}^{-1}$) | 2.33±1.08 | 1.58±1.03 |
| NP molar ratio | 9.89±11.13 | 15.14±7.83 |
| After 4-week exposure (ARO treatments) | | |
| Terbutylazine | - | - |
| Pirimicarb | 12.4±0.7 | 13.7±0.7 |
| Tebuconazole | 31±8.1 | 34±2.5 |
| Copper | 13.1±6.1 | 13.5±7.6 |

* 0.013 mg L⁻¹ before P fertilization. See text for details. Cond = conductivity, Alk. = Alkalinity, DIN = dissolved inorganic nitrogen.

Table S5. Statistical details for individual and combined stressor effects on the different functional groups.

| | | Individual effects | Interactions | | | | | |
|--|-------------------------|-----------------------------|------------------------------|-------------|---------------|-----|-------------|-------|
| | | T | A | W | T*A | T*W | A*W | T*A*W |
| Endpoints | Test | <i>p-value</i> | | | | | | |
| Macrophytes | | | | | | | | |
| MS biomass | 3-way ANOVA | 1.25×10⁻⁵ | 2.66×10⁻¹⁰ | 0.467 | 0.1 | 0.5 | 0.7 | 0.6 |
| PF biomass | 3-way ANOVA | 0.1 | 0.5 | 0.4 | 0.5 | 0.5 | 0.4 | 0.4 |
| EN biomass | 3-way ANOVA | 0.1 | 0.05 | 0.01 | 0.04 | 0.1 | 0.2 | 0.4 |
| Total macro-phyte biomass | 3-way ANOVA | 0.0001 | 1.59×10⁻¹⁰ | 0.07 | 0.1 | 0.3 | 0.6 | 0.4 |
| Microalgae | | | | | | | | |
| Biofilm_chla | 3-way ANOVA | 0.2 | 3.90×10⁻⁶ | 0.7378 | 0.01 | 0.6 | 0.02 | 0.7 |
| Phytoplankton chla | 3-way ANOVA | 0.01 | 0.02 | 0.1 | 0.0007 | 0.2 | 0.6 | 0.1 |
| Shifts in primary producer abundance | | | | | | | | |
| Macrophyte, phytoplankton and periphyton C | PERMANOVA | 0.01 | 0.001 | 0.5 | 0.01 | 0.5 | 0.5 | 0.6 |
| Consumers | | | | | | | | |
| LS_length | 3-way ANOVA | 0.005 | 2.08×10⁻⁶ | 0.9 | 0.06 | 0.8 | 0.5 | 0.05 |
| DM_number_w1 | 3-way ANOVA | 0.02 | 2.56×10⁻⁵ | 0.4 | 0.4 | 0.9 | 0.9 | 0.7 |
| DM_number_w2 | 3-way ANOVA | 0.1 | 0.01 | 0.7 | 0.08 | 0.6 | 0.7 | 0.06 |
| DM_number_w3 | 3-way ANOVA | 0.1 | 0.01 | 0.8 | 0.08 | 0.6 | 0.8 | 0.06 |
| DM_number_w4 | Kruskal-Wallis rank sum | 0.2 | 3.00×10⁻³ | 0.11 | | | | |
| DM_biomass | 3-way ANOVA | 0.09 | 3.00×10⁻⁴ | 0.9 | 0.2 | 0.4 | 0.2 | 0.4 |
| Optical density | | | | | | | | |
| OD_663_w1 | Kruskal-Wallis rank sum | 0.03 | 0.9 | 0.4 | | | | |
| OD_663_w2 | 3-way ANOVA | 0.7 | 0.04 | 0.5 | 0.08 | 0.5 | 1 | 0.9 |
| OD_663_w3 | Kruskal-Wallis rank sum | 0.5 | 9.15×10⁻⁶ | 0.2 | | | | |
| OD_663_w4 | Kruskal-Wallis rank sum | 0.2 | 2.17×10⁻⁵ | 0.2 | | | | |

Table S5. Statistical details for individual and combined stressor effects on the different functional groups (contd).

| | | Individual effects | Interactions | | | | | |
|--|--|--------------------|--------------|---|-----|-----|-----|-------|
| | | T | A | W | T*A | T*W | A*W | T*A*W |

| Endpoints | Test | <i>p-value</i> | | | | | | |
|-------------------|-------------------------|-----------------------|------------------------|------------------------|-------|------|-------|-----|
| Conductivity | | | | | | | | |
| Conductivity_w0 | Kruskal-Wallis rank sum | 0.3 | 0.6 | 8.47×10 ⁻⁸ | | | | |
| Conductivity_w1 | 3-way ANOVA | 0.2 | 6.21×10 ⁻⁸ | 1.09×10 ⁻⁵ | 0.02 | 0.1 | 0.009 | 0.4 |
| Conductivity_w2 | 3-way ANOVA | 0.01 | 0.4 | 8.65×10 ⁻¹⁰ | 0.002 | 0.9 | 0.001 | 0.2 |
| Conductivity_w3 | 3-way ANOVA | 0.0006 | 0.2 | 2.53×10 ⁻⁶ | 0.09 | 0.8 | 0.03 | 0.8 |
| Conductivity_w4 | 3-way ANOVA | 6.94×10 ⁻⁵ | 0.1 | 1.80×10 ⁻⁷ | 0.02 | 0.4 | 0.09 | 0.9 |
| Oxygen saturation | | | | | | | | |
| O2_w0 | 3-way ANOVA | 0.4 | 0.8 | 0.1 | 0.9 | 0.1 | 0.8 | 0.6 |
| O2_w1 | 3-way ANOVA | 0.7 | 0.03 | 0.5 | 0.3 | 0.08 | 0.9 | 0.4 |
| O2_w2 | 3-way ANOVA | 0.04 | 5.03×10 ⁻⁹ | 0.2 | 0.9 | 0.6 | 0.03 | 0.4 |
| O2_w3 | Kruskal-Wallis rank sum | 0.2 | 0.2 | 0.2 | | | | |
| O2_w4 | Kruskal-Wallis rank sum | 0.2 | 0.2 | 0.2 | | | | |
| pH | | | | | | | | |
| pH_w0 | 3-way ANOVA | 0.4 | 0.5 | 0.02 | 0.4 | 0.2 | 0.8 | 0.4 |
| pH_w1 | 3-way ANOVA | 0.0003 | 0.001 | 0.03 | 0.5 | 0.09 | 0.3 | 0.2 |
| pH_w2 | 3-way ANOVA | 0.01 | 1.85×10 ⁻¹² | 0.5 | 0.5 | 0.9 | 0.1 | 0.6 |
| pH_w3 | Kruskal-Wallis rank sum | 0.05 | 0.0003 | 0.04 | | | | |
| pH_w4 | Kruskal-Wallis rank sum | 0.002 | 0.5 | 0.2 | | | | |

Note: MS = *M. spicatum*, PF = *Potamogeton perfoliatus*, EN = *E. nuttallii*, LS = *L. stagnalis*, DM = *D. magna*, OD = optical density. T = temperature, A = ARO, W = water.

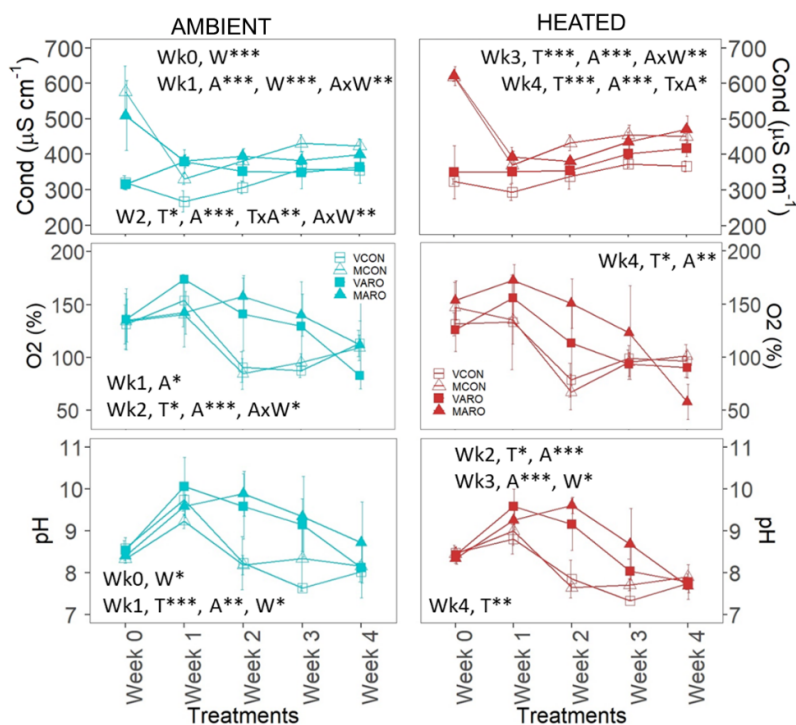


Figure S1. MICROCOSMS. Weekly measures of water physico-chemistry in the ambient (**left**, blue lines and symbols) and heated (**right**, red lines and symbols) microcosms. VCON = Volvic control; MCON = Munich well water control. ARO; VARO = Volvic ARO; MARO = Munich well water ARO. A = ARO, T = Temperature, W = Water. Means \pm SD, $n = 5$. Statistical differences between treatments are shown per week (Wk1, 2, 3, or 4) and factor (A, T or W) or interactions between factors. Significance levels shown as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

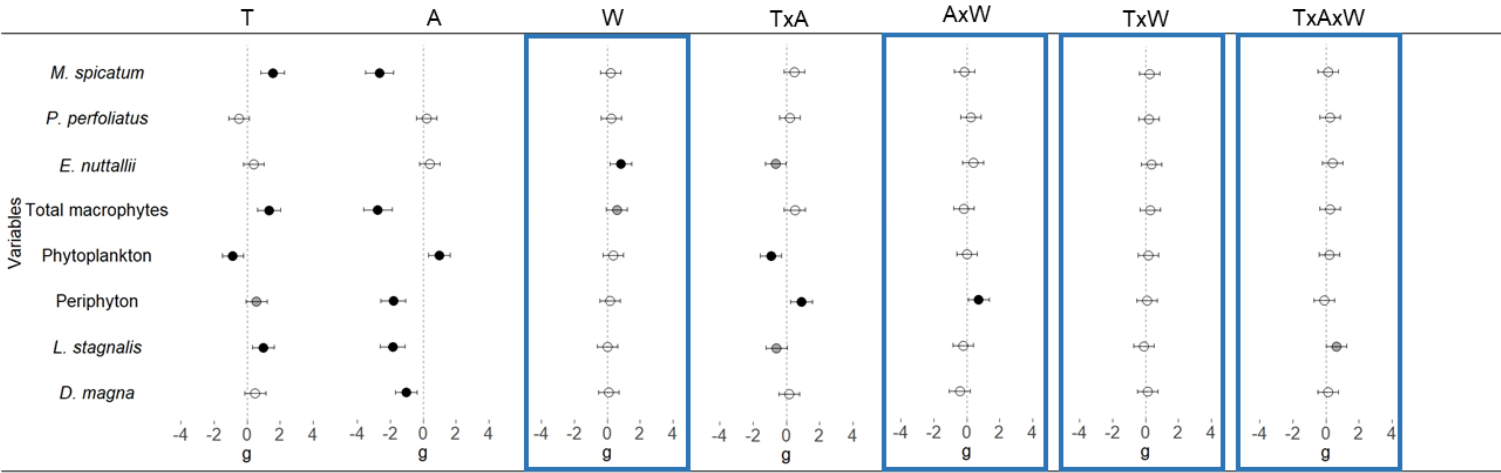


Figure S2. Effect sizes (Hedges' $g \pm 95\%$ confidence interval) of individual stressors and stressor interactions on the key functional groups at the end of the experiment, calculated from the F -statistics derived from the ANOVA. No effects are inferred when confidence intervals cross the zero line. White symbols: non-significant effects (>0.09), grey symbols: marginal effects ($0.05\text{--}0.09$), black symbols: significant effects (<0.05). A = ARO, T = Temperature, W = Water. Water effects and its interactions with temperature and ARO or both are outlined in blue.

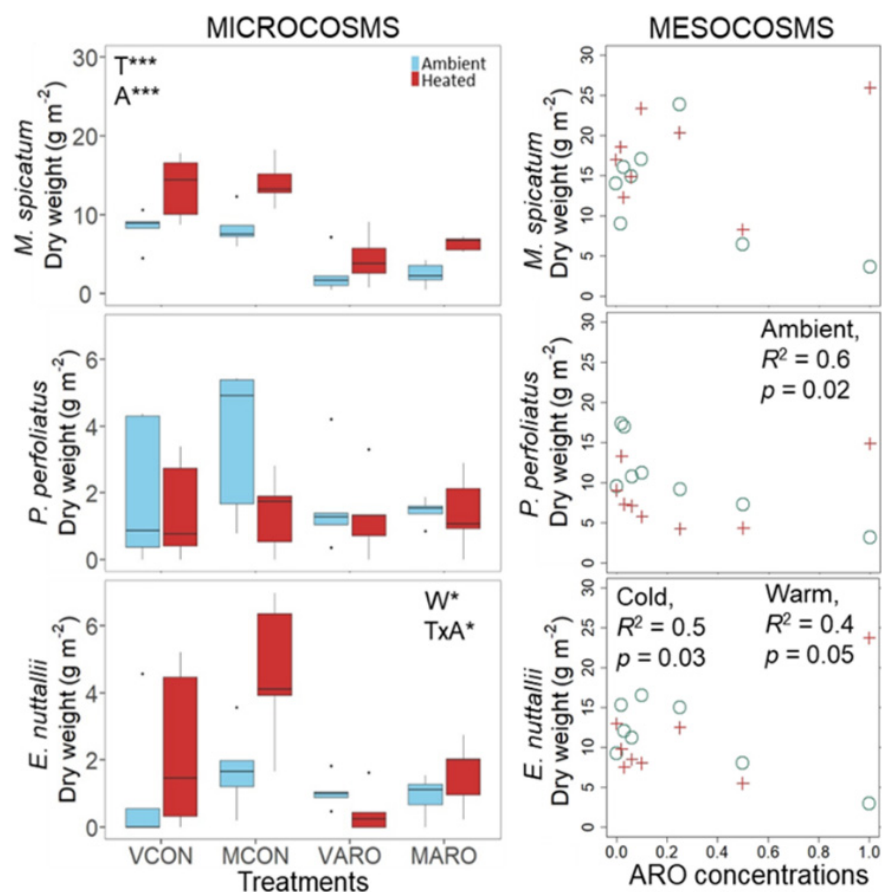


Figure S3. Stressor effects on macrophytes in the micro- and mesocosms. Microcosms: VCON = Volvic control; MCON = Munich well water control. ARO; VARO = Volvic ARO; MARO = Munich well water ARO. A = ARO, T = Temperature, W = Water. Box plots of 5 replicates showing median, 25 and 75% percentiles, lowest and highest whiskers (as $Q1 - [1.5 \times IQR]$ and $Q3 + [1.5 \times IQR]$, respectively), and outliers (dots). Mesocosms: R^2 and p values from linear regressions. For both experiments, significance levels shown as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

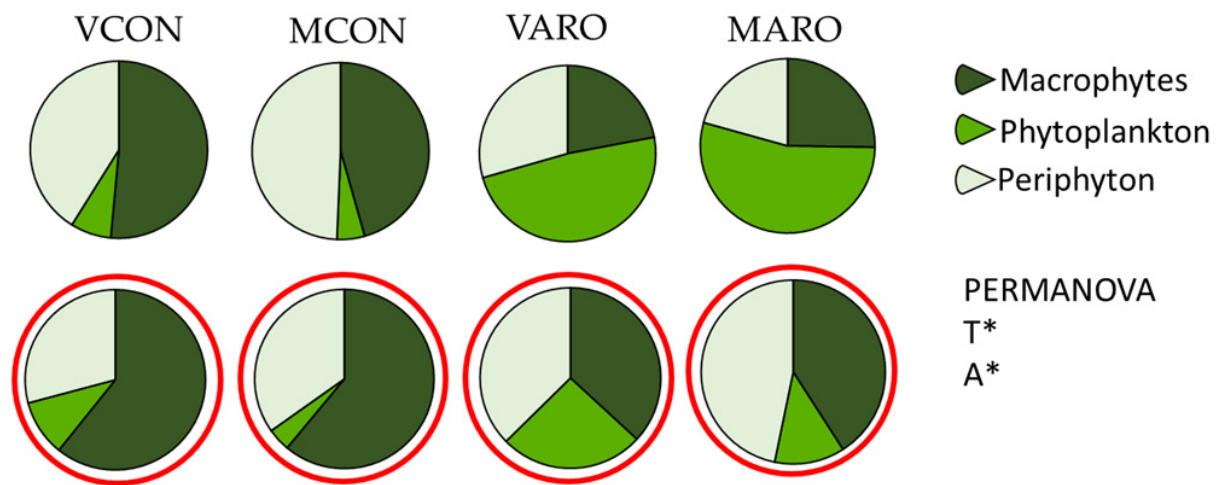


Figure S4. MICROCOSMS. Relative proportion of primary producers when exposed to the ARO in Volvic and Munich well water at the two temperatures. VCON = Volvic control; MCON = Munich well water control. ARO; VARO = Volvic ARO; MARO = Munich well water ARO. A = ARO, T = Temperature, W = Water. Significance levels shown as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Heated treatments are represented by a red circle around the pie charts.

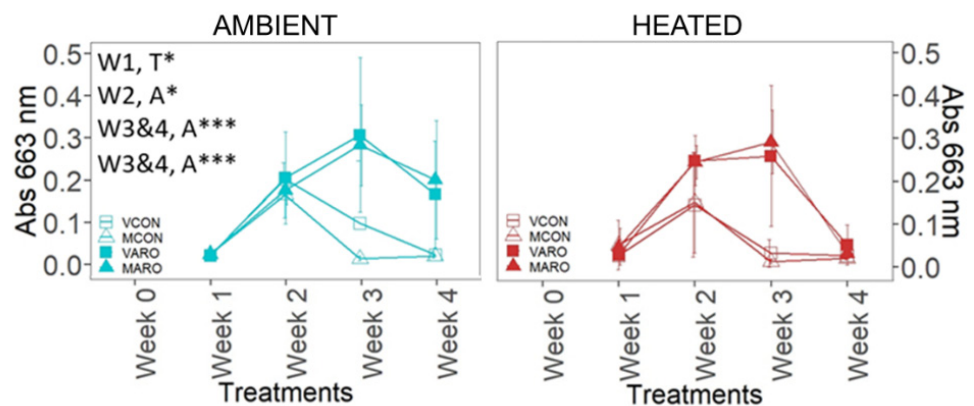


Figure S5. MICROCOSMS. Weekly measures of absorbance of water at 663 nm in the ambient (blue) and heated (red) mesocosms. Blue represents the unheated microcosms and red indicates the heated. ARO; VARO = Volvic ARO; MARO = Munich well water ARO. A = ARO, T = Temperature, W = Water. Means \pm SD, $n = 5$. Statistical differences between treatments are shown per week (Wk1, 2, 3, or 4) and factor (A, T or W) or interactions between factors. Significance levels shown as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

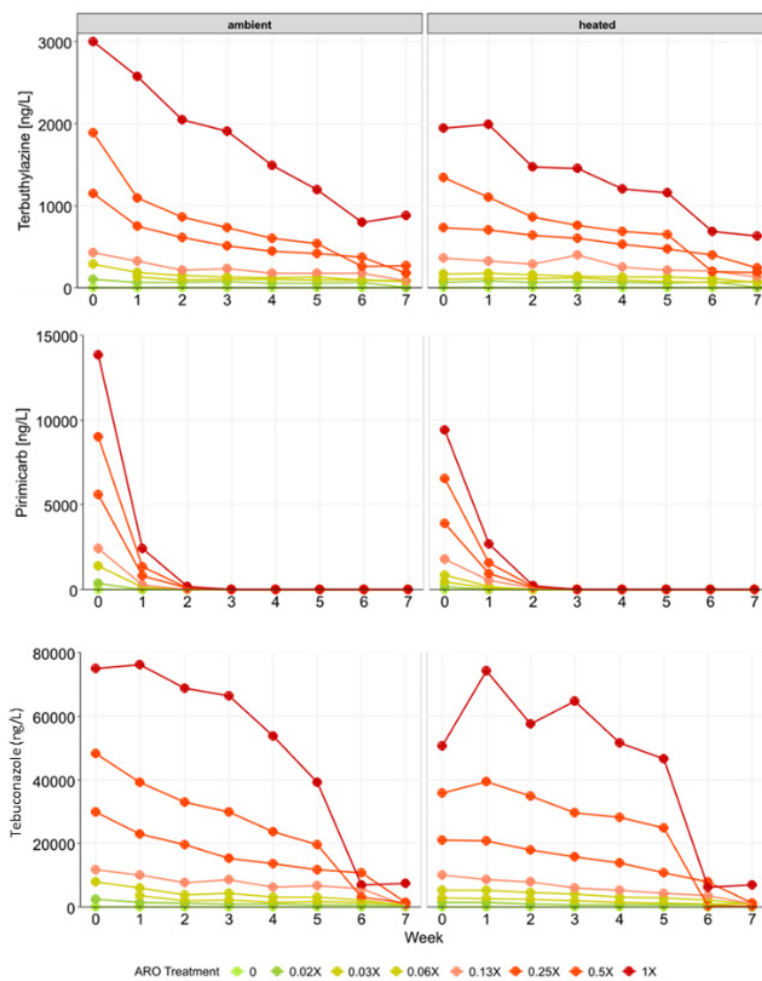


Figure S6. MESOCOSMS. Pesticide concentrations measured over 8 weeks.

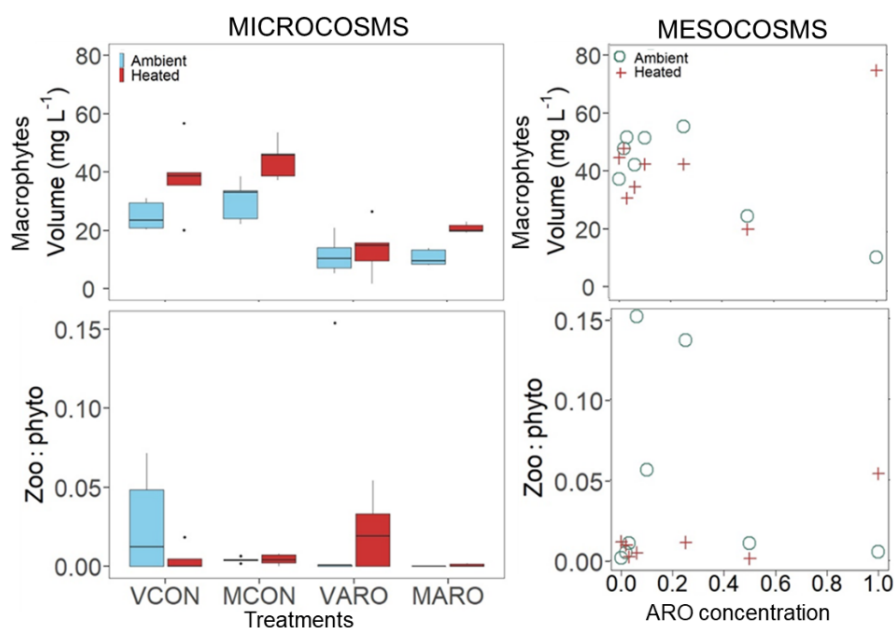


Figure S7. Biomass per volume of macrophytes and zooplankton:phytoplankton ratios (zoo:phyto; mass ratios) in the microcosms (left) and mesocosms (right). Microcosms: VCON = Volvic control; MCON = Munich well water control. ARO; VARO = Volvic ARO; MARO = Munich well water ARO. Box plots of 5 replicates showing median, 25 and 75% percentiles, lowest and highest whiskers (as $Q1 - [1.5 \times IQR]$ and $Q3 + [1.5 \times IQR]$, respectively), and outliers (dots).