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Species-Specific Responses of Submerged Macrophytes to Simulated Extreme Precipitation: A Mesocosm Study

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Abstract: More frequent extreme climate events (e.g., extreme precipitation) are to be expected in the future, and such events may potentially have significant effects on freshwater ecosystems. In the present mesocosm study, the effects of simulated extreme precipitation on submerged macrophytes were evaluated for three different macrophyte community (MC) treatments (MC1, MC2 and MC3). MC1 consisted of only *Vallisneria denseserrulata*, while MC2 and MC3 included three and six species of various growth forms. Two treatments of extreme precipitation (EP) were simulated—an extreme treatment (E) simulating a sudden increase of water level from 75 cm to 150 cm within one day and a gradual treatment (G) simulating an increase to the same water level within 3 months, combined with two control treatments. Total macrophyte community biomass was resilient to the EP and MC treatments, while species-specific variations in responses, in terms of biomass, maximum height, and sexual reproduction, were found. For instance, E led to earlier flowering of *Potamogeton lucens* and production of more flowers, while it had adverse effects on the flowering of *Ottelia alismoides*. We conclude that freshwater ecosystems with high coverage of submerged macrophytes may be overall resilient to extreme precipitation under nutrient-limited conditions, especially communities with diverse growth forms.

Keywords: climate change; *Vallisneria*; *Ottelia*; *Potamogeton*; charophyte; *Radix swinhoei*

1. Introduction

The Intergovernmental Panel on Climate Change predicts more frequent extreme precipitation events in the future with potential strong influence on freshwater ecosystems [1,2]. The effects of extreme precipitation in shallow lakes may both lead to a sudden increase of water levels and an additional input of nutrient. Studies focusing on either extreme precipitation or nutrient pulses have revealed that a sudden water level increase after extreme rainfall significantly influences primary producers in freshwater ecosystems [3–5] and that pulse loading of nitrogen can induce marked changes in macrophyte and phytoplankton communities [6]. So far, studies on the combined effects of extreme rainfall-mediated increases in water level and nutrient pulses are scarce. A recent study reported that water level increase combined with a nutrient pulse had no negative effect on the growth

of *Vallisneria denseserrulata*, while a water level increase without extra nutrient loading had, suggesting that nutrients somehow counteracted the effect of the water level increase [7].

Submerged macrophytes are dominant primary producer in shallow clear lakes and they play an important structuring role for the community structure and ecosystem function [8]. For example, macrophytes compete with phytoplankton for light and nutrients [9], and they create and maintain a clear water state [10], but their ecological effect in shallow lakes may differ among growth forms [11–14]. It has been hypothesised that the clear-water state dominated by charophytes differs from the clear-water state dominated by angiosperms as bottom-up mechanisms (i.e., food webs controlled by nutrient availability and primary producers) characterise the charophyte-dominated state, while top-down mechanisms (i.e., food webs controlled by predators) are more important in an angiosperm-dominated state [13]. However, the effects of angiosperms on contrasting growth forms may also differ. Angiosperm species may be categorised into slow-growing and canopy-forming types [7,12,14,15]; slow-growing angiosperms increase slowly in height and allocate most of their biomass near the lake bottom, while the canopy-forming types distribute large proportions of their biomass near the surface. *Vallisneria* spp. are considered as a typical slow-growing species, while *Potamogeton lucens* (*P. lucens*) is a typical canopy-forming species, and other species are found between these two extremes. Macrophyte communities with diverse growth forms may affect light attenuation in the water column differentially, with implications for the communities of periphyton and macroinvertebrates and, in consequence, the growth of macrophyte species in the entire community [12,16,17]. Moreover, many macrophyte species, like *P. lucens* and *Ottelia alismoides* (*O. alismoides*), which both have broad leaves and a strong affinity for bicarbonate usage, typically have different pH levels on the upper and lower leaf side depending on light conditions (polarised leaves, e.g., [18,19]). This effect, which is strongly driven by photosynthesis [20], induces depletion of free CO_2 near the adaxial side of the phyllosphere. Similarly, charophytes are experts in bicarbonate usage and during photosynthesis, one long cell of the thalli is segmented into several adjacent alkaline and acid zones (so-called multi-polarisation), which also affects the pH and alkalinity in the nearby area [21,22]. This could potentially induce carbon limitation of periphyton growth and of nearby submerged macrophytes [23], especially for the species that mainly use free CO_2 as their carbon supply (e.g., *Cabomba caroliniana* according to [24]).

Observed difference in responses of submerged macrophytes to extreme events such as extreme heat waves [25–27] may in part reflect differences in growth form. Also, the effects of extreme water level changes may depend on growth forms [14,25,26]. The Otteliid species *O. alismoides*, for example showed no significant changes in total biomass and root biomass between static and fluctuating water level simulations [28], while *Myriophyllym spicatum* demonstrated stronger morphological responses (e.g., longer shoots, more branches) and reduced biomass compared with e.g., *Hydrilla verticillata* at water level fluctuations of 60 cm or larger [29]. In contrast, among the slow-growing types, *Vallisneria* spp. can adapt to increasing water levels through photosynthetic adjustment [30], and it thus does not exhibit significant shoot elongation when exposed to high water levels and low light availability [31].

Here, we aim to investigate whether the responses of submerged macrophytes to simulated extreme precipitation were species-specific and related to growth form under phosphorus-limited conditions, considering simultaneously two aspects of extreme precipitation (water level increase and nutrient pulse). We hypothesised that (1) due to many positive feedbacks from macrophyte communities (e.g., uptake of excessive nutrients and competition for light with phytoplankton [8,32]), the clear-water state dominated by submerged macrophytes would be resilient to an extreme precipitation event (high nutrient input and a sudden water level increase); (2) macrophyte species specific responses would vary with the growth form combination of the entire macrophyte community as the canopy-forming types compete better for light than the slow-growing types.

2. Materials and Methods

2.1. Experimental Materials

The submerged macrophytes used in the experiment grew naturally in small clear-water ponds in Wuhan Botanical Garden (114.43° E, 30.54° N). The slow-growing *Vallisneria denseserrulata* (*V. denseserrulata*) is a perennial submerged species that maintains its aboveground biomass in winter. The species is mainly found in Central-Southern China. It is capable of using bicarbonate but does not form polarised leaves. Likewise, *Chara sp.* is a typical slow-growing species, showing multi-polarisation in its thalli. The two species differed in plant height, *Chara sp.* being much shorter (approximately 30 cm) than *V. denseserrulata* (approximately 50~70 cm) in the same ponds.

Potamogeton lucens and *Ottelia alismoides* are two typical canopy-forming species, which both are bicarbonate users. *P. lucens* is a common species in clear-water lakes in the mid-low reaches of Yangtze River and in Yunnan Province, China, while *O. alismoides*, which used to be widely distributed, is no longer a commonly-found species in recent field surveys [7].

Hydrilla verticillata (*H. verticillata*) and *Cabomba caroliniana* (*C. caroliniana*) distribute their biomass more or less evenly in the water column and represent a growth type in-between the two slow-growing and canopy-forming extremes. *H. verticillata* is a bicarbonate user with polarised leaves during photosynthesis, while *C. caroliniana* only uses free CO_2 as carbon supply and is an invasive species in China [24].

2.2. Experimental Design

Three contrasting communities of submerged macrophytes were established in the macrophyte community (MC) treatment, and four different scenarios of extreme precipitation (EP) were simulated. In total, 36 concrete mesocosms (four EP treatments and three MC treatments, each treatment with three replicates, each mesocosm with nine pots), 1 m long, 1 m wide and 1.5 m deep, including a total of 324 pots, were used in the experiment.

To mimic the species composition of macrophyte communities in natural lakes, three different macrophyte communities composed of macrophytes of various growth forms were established (hereafter termed MC1, MC2 and MC3). MC1 consisted only of *V. denseserrulata* (Vd). In this treatment, nine pots (27 cm top diameter × 22 cm bottom diameter × 14 cm height) were placed in one mesocosm, each pot holding two individuals with an initial fresh weight of 1.5 g. MC2 included three species: Vd, *P. lucens* (Pl) and *H. verticillata* (Hv). Three pots of each species, four Hv, three Pl and two Vd with equal initial fresh weights (1.5 g) in each pot, were placed randomly in one mesocosm. Besides the three species of MC2, MC3 contained *C. caroliniana*, *Chara sp.* and *O. alismoides*, and three individuals of *C. caroliniana* and *O. alismoides* as well as a small clump of *Chara sp.* The former three species were represented by two pots for each species and the latter three by one pot for each species. In total, nine pots, each with an initial fresh weight of 1.5 g were planted in each mesocosm, ensuring a similar initial biomass for all mesocosms.

During the one-month pre-cultivation period, the water level was set to 75 cm. The control (C) maintained the same water level throughout the experiment. An extreme treatment (E) simulated an extreme precipitation event with a sudden increase in water level to 150 cm within one day. A gradual treatment (G) simulated a gradual increase in water level to 150 cm within three months, 25 cm in a day but adjusted monthly. Tap water (total nitrogen (TN): 2.2 ± 0.5 (standard deviation (SD) here and elsewhere) mg L^{-1}, total phosphorus (TP) 45 ± 12 μg L^{-1}) was used to simulate extreme precipitation, and the increase in water level was obtained by adding tap water until the required level was reached. To separate the effects of nutrient input from those of the sudden water level increase, an extra treatment with a 75 cm stable water level but extra nutrient loading (C_N) was designed. Apart from C, the other three treatments received the same loading of nitrogen and phosphorus in the form of chemicals (KNO_3 and KH_2PO_4).

By adding lake water from Donghu Lake, the water levels were initially kept stable at 75 cm, and nine pots of submerged macrophytes (community composition arranged according to the experiment design defined above) were placed in each mesocosm and pre-cultivated for one month from 13 July 2015. Two approximately 3 cm-long male *Gobiocypris rarus*, an endemic Chinese planktivore feeding on zooplankton, were released in each mesocosm. Moreover, five approximately 1 cm-long *Radix swinhoei* were added and acted as the main macro-invertebrates in the system [33].

The experiment lasted from August 2015 to May 2016. On 19 August, a sudden increase in the water level to 150 cm was mimicked in the E treatment by adding tap water (pulse input of approximately 1600 mg N m^{-2} and 37.5 mg P m^{-2}). The water level in the G treatment was increased by 25 cm on the same day, and the nutrient loadings of C_N and G were also maintained at levels similar to those in E by adding KNO_3 and KH_2PO_4. The water level in G was increased by 25 cm monthly to 150 cm during the following two months, and a similar final nutrient loading as in C_N and E was achieved by the addition of chemicals (approximately 680 mg N m^{-2} and 13.7 mg P m^{-2}). Tap water was added to maintain the water level in all the treatments afterwards, resulting only in negligible nutrient input [6,34]. Details on the experiment design are given in Figure S1 in the Supplementary Materials.

2.3. Sampling Schedule and Measurement of Plant Traits and Water Physico-Chemical Parameters

On 12 August, water samples for phytoplankton biomass and water chemistry measurements were taken before the EP treatment to record the initial conditions of phytoplankton chlorophyll *a* (Chl*a*), total nitrogen (TN), nitrate (NO$_3$-N) and total phosphorus (TP). From 19 August to 21 November, weekly water samples were taken to explore the detailed dynamics of the effects of EP in different macrophyte communities. From 6 December to 7 May the next year, water samples were taken biweekly for determination of water chemistry variables and phytoplankton biomass. The water samples were taken in different layers of the water column with a 1 L plastic tube sampler and were thoroughly mixed before analysis, thus representing the entire water column. Water samples of 1 L for phytoplankton Chl*a* analysis were filtered on a GF/C filter (Whatman, Maidstone, UK) following ethanol extraction, and the filtered water was used for NO$_3$-N determination according to the phenol disulphonic acid method [35]. Water samples for TN and TP analysis were unfiltered and determined using the spectrophotometric methods after digestion with $K_2S_2O_8$ solution [35].

Dissolved oxygen (DO), temperature (T), light attenuation coefficient (Kd), pH and alkalinity were determined monthly. DO and T were measured using a ProODO Optical Dissolved Oxygen Instrument (YSI, Ohio, USA) at 30 cm depth at the same time of the day (2 p.m. to 4 p.m.). Kd was calculated using the light attenuation at 30 cm below the surface applying the formula in [36] based on the measurements of a Li-192 underwater quantum sensor and data-logger Li-1400 (LI-COR, Nebraska, USA). Water samples of 250 ml were collected and sealed in blue cap bottles for pH and alkalinity analysis, and alkalinity was determined by titration using 0.1 mmol L^{-1} HCl within four hours after sample collection. Theoretical daylight length (DL) was estimated for each month based on the geographical location of the experiment sites. The data of T and DL are provided as background information on environmental conditions in Figure S2 in the Supplementary Materials.

Flower numbers were observed and recorded for *V. denseserrulata*, *P. lucens* and *H. verticillata* during the flowering season (from September to November).

At the end of the experiment, all the macrophytes in the pots, both above and below ground, were harvested and cleansed in tap water. Maximum plant height and biomass were recorded. For *V. denseserrulata* and *P. lucens*, the second mature leaf of a randomly-chosen individual was used for determination of epiphyton biomass. The leaf was carefully sampled, placed into a sealed plastic bag and stored in a cooling box. In the laboratory, tap water was added to the sealed plastic bag, and after thorough shaking for 1 min the washed-off water was filtered on GF/C filters for determination of the chlorophyll *a* content [37]. All remaining leaves were carefully cleaned and, together with the first leaf, divided into three groups indicating different extents of damage: intact (no holes or damages

at the leaf edge), minor damage (≤3 holes or leaf edge damages) and major damage (>3 holes or leaf edge damages). Snail biomass and abundance were recorded by using a 500 μm invertebrate net to collect all the snails in each mesocosm before and after draining the mesocosm; in total, over 1000 snail individuals were sampled. Filamentous algae were collected by a 20 cm scraper to remove all the filamentous algae on the 20 cm wide strap at the centre of the walls (the same position for all mesocosms) from the bottom to the surface; the scraping was carefully undertaken after draining the mesocosm to minimise sampling loss. Filamentous algae biomass was determined after drying the algae at 80 °C for 48 h followed by weighing.

2.4. Statistical Analysis

For the data obtained on 12 August, two-way ANOVA was used to assess background conditions before the extreme precipitation using MC and EP as the two main factors. Since the sampling interval shifted from one to two weeks during the course of the experiment, a linear mixed model was considered proper to handle the dataset [38]. For the water physico-chemical variables recorded during the experiment, linear mixed models (LMMs) were used to identify the effects of the MC and EP treatments on each parameter. In the model, MC and EP were set as fixed factors, and sampling time and tank id were used as random factors based on the function 'lmer' in package 'lmerTest'. Residual plots were used to check for normality and homogeneity of variance by visual inspection. Post-hoc Tukey tests were conducted using the package 'multicomp'. For the data on plant traits, snail biomass, filamentous algae and epiphyton biomass recorded at the end of experiment, two-way ANOVA was applied to explore the effects of the MC and EP treatments. For correlation analyses between snail biomass (or abundance) and periphyton biomass (or submerged macrophyte leaf intactness) we used the function 'chart.Correlation' in package 'PerformanceAnalytics'. Data were log transformed to satisfy the assumption of normality and homogeneity of variance, if needed. All statistical analyses were performed using R 3.5.3 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Water Physico-Chemical Parameters

Prior to the EP treatment (on 12 August), TN, NO_3-N, TP, phytoplankton Chl*a*, alkalinity, pH, Kd and DO did not differ among the three MC or four EP treatments after one month of pre-cultivation in July, excepting pH that was 8.20 ± 0.13 in MC1, which was slightly higher than in MC2 (8.04 ± 0.12) and MC3 (8.08 ± 0.15) (for pH between MC, F = 3.87, $p < 0.05$).

TP generally ranged within 20~25 μg L^{-1} except on the first sampling event after the simulated extreme precipitation, where it was higher in C_N and G than in C (Table 1, Figure 1). TN and NO_3-N did not differ among the three macrophyte community treatments, but for the four EP treatments, TN and NO_3-N were highest in C_N, being 1.77 ± 0.72 mg L^{-1} and 1.27 ± 0.68 mg L^{-1}, respectively, and lowest in C, being 0.64 ± 0.23 mg L^{-1} and 0.16 ± 0.13 mg L^{-1}, respectively.
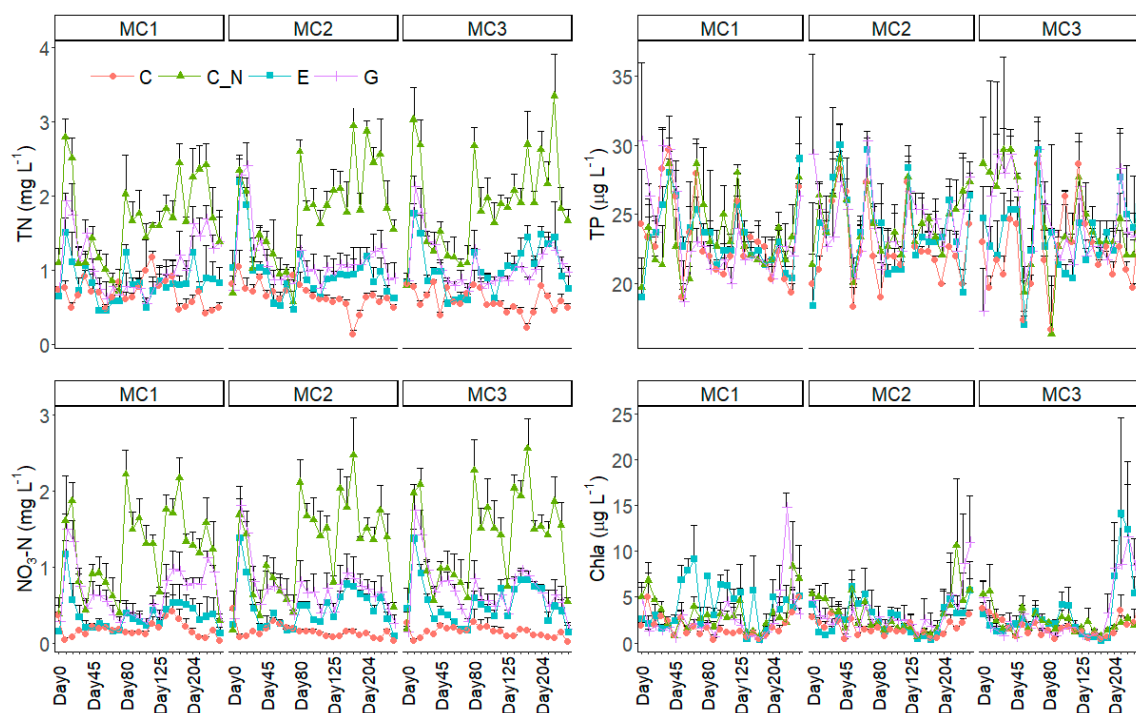
Phytoplankton Chl*a* fluctuated during the experiment and did not differ among the four treatments in the post-hoc test (Table 1, Figure 1). After dividing the dataset into three subsets consisting of each MC treatment, LMM revealed that phytoplankton Chl*a* was lower in C than in E in subset MC1 (F = 4.18, $p < 0.05$) but not in subset MC2 or MC3 (F < 1.08, $p > 0.05$ for both).

Kd was lowest in MC1 holding only *V. denseserrulata* and also lower in the two water level increase treatments (E and P) than in C and C_N (Table 1, Figure 2). Alkalinity was significantly lower, and pH was higher, in E than in the other three EP treatments, but DO was only significantly different (higher) in E than in C and C_N and did not differ from G. All three indicators, Kd, alkalinity and DO, did not differ among the three macrophyte communities.

Table 1. Statistical summary of results of the linear mixed models (LMMs) on the effects of macrophyte community (MC: MC1, MC2 and MC3) and extreme precipitation (EP: control (C), extra nutrient loading (C_N), extreme treatment (E) and gradual treatment (G)) on physico-chemical parameters including total phosphorus (TP), total nitrogen (TN), nitrate (NO_3-N), phytoplankton Chl*a*, alkalinity, pH, light attenuation coefficient (Kd) and dissolved oxygen (DO). The post-hoc test was conducted by the Tukey method. NS = not significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Indicators	LMMs F Values and Significance (Sig.)			Post-Hoc Test	
	MC	EP	Interaction	MC	EP
TP	1.47, NS	4.82, **	1.20, NS		C< C_N, G
TN	0.73, NS	62.62, ***	0.58, NS		C< E, G < C_N
NO_3-N	0.51, NS	45.62, ***	0.20, NS		C< E < G < C_N
Phytoplankton Chl*a*	0.30, NS	3.26, *	0.51, NS		
Kd	10.46, ***	15.34, ***	1.01, NS	MC1 < MC2, MC3	E, G < C, C_N
Alkalinity	1.03, NS	4.37, *	1.11, NS		E < C, G, C_N
pH	0.63, NS	6.15, **	0.62, NS		E > C, G, C_N
DO	2.77, NS	4.26, *	0.58, NS		E > C, C_N

Figure 1. The changes in physico-chemical parameters (mean + SD) including total nitrogen (TN), total phosphorus (TP), nitrate (NO_3-N) and phytoplankton chlorophyll *a* (Chl*a*) during the experiment for the three macrophyte community treatments (MC1, MC2 and MC3) and the four extreme precipitation treatments (EP: C, C_N, E and G). For clarity, SD values are only shown in one direction.

3.2. Macrophyte Communities

The total biomass of macrophytes did not differ between the three MC or the four EP treatments, but at the species level contrasting responses emerged (Table 2, Figure 3). The biomass (per pot) and maximum plant height of *V. denseserrulata* were significantly larger in MC1 than in the other two macrophyte communities (Figure S3). In contrast, *P. lucens* had larger maximum plant height in the two water level increase treatments (E and G) than in C_N, but the biomass of *P. lucens* did not respond significantly to the MC or EP treatments. For *H. verticillata*, total biomass and maximum plant height did not differ between MC2 and MC3 or among the EP treatments. In MC3, *C. caroliniana* had lowest biomass in E, while *Chara sp.* had the largest biomass and maximum plant height in C.
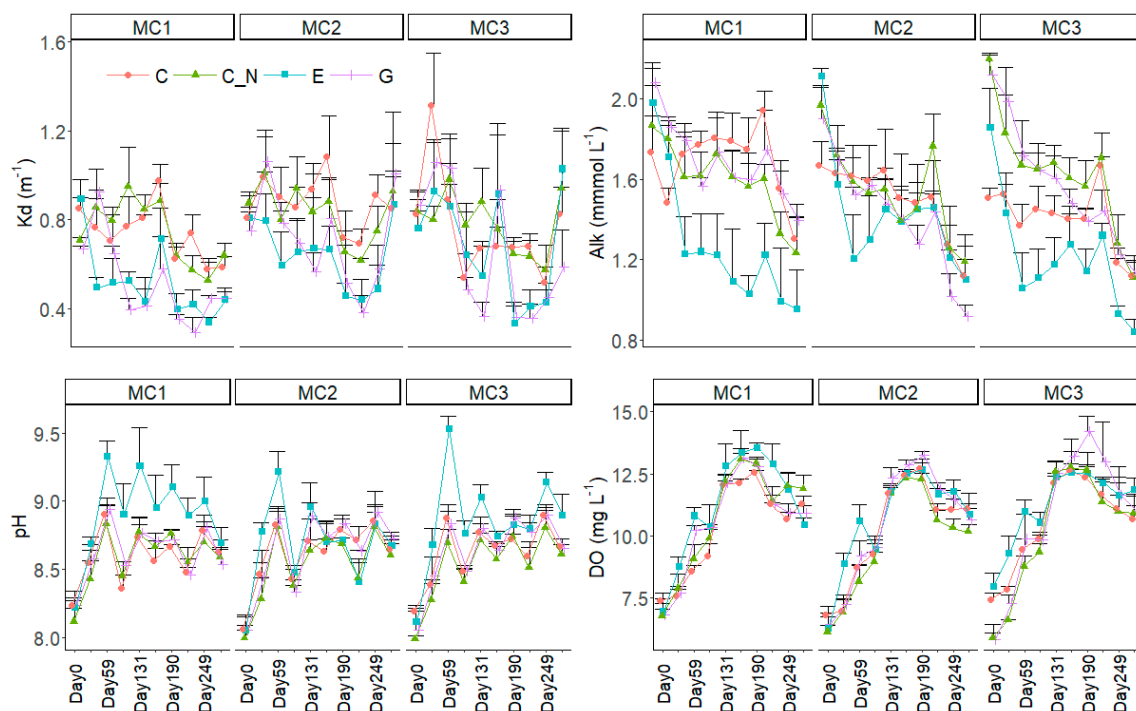
Figure 2. Changes in physico-chemical parameters (mean + SD) including light attenuation coefficient (Kd), alkalinity (Alk), pH and dissolved oxygen (DO) during the experiment for three macrophyte community treatments (MC1, MC2 and MC3) and the four extreme precipitation treatments (EP: C, C_N, E and G). For clarity, SD values are only shown in one direction.

Table 2. Statistical summary of two-way ANOVA on the effects of the macrophyte community (MC: MC1, MC2 and MC3) and extreme precipitation (EP: C, C_N, E and G) on plant biomass and morphology at the end of the experiment. The post-hoc test was conducted by the Tukey method. NS = not significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Species/Indicators		Two-Way ANOVA			Post-Hoc Test	
		MC F Values, Sig.	EP F Values, Sig.	Interaction F Values, Sig.	MC	EP
Macrophyte community	Total biomass	0.49, NS	0.74, NS	0.09, NS	-	-
Vallisneria denseserrulata	Total biomass	23.10, ***	1.09, NS	0.80, NS	MC1 > MC2, MC3	-
	Maximum height	4.97, *	1.15, NS	0.87, NS	MC1 > MC2, MC3	-
Potamogeton lucens	Total biomass	0.05, NS	0.25, NS	0.29, NS	-	-
	Maximum height	0.01, NS	4.49, *	0.62, NS	-	C_N < E, G
Hydrilla verticillata	Total biomass	0.03, NS	0.18, NS	0.55, NS	-	-
	Maximum height	0.07, NS	0.76, NS	0.19, NS	-	-
Cabomba carolinana	Total biomass	Not tested	4.452, *	Not tested	-	E < G, C, C_N
	Maximum height	Not tested	4.31, *	Not tested	-	E < G
Chara sp.	Total biomass	Not tested	7.84, **	Not tested	-	C > G, E, C_N
	Maximum height	Not tested	14.37, **	Not tested	-	C > G, E, C_N

3.3. The Flowering Phase of V. denseserrulata, O. alismoides *and* P. lucens

V. denseserrulata had the largest number of female flowers in both October and November (F > 6.92, $p < 0.01$ for MC; Figure 4), reaching 2.9 ± 3.3 per tank in November in MC1. The flowering number did not differ among the four EP treatments during the experiment (F < 2.42, $p > 0.05$). However, the species did not flower in MC2 and MC3 at any time during the experiment.
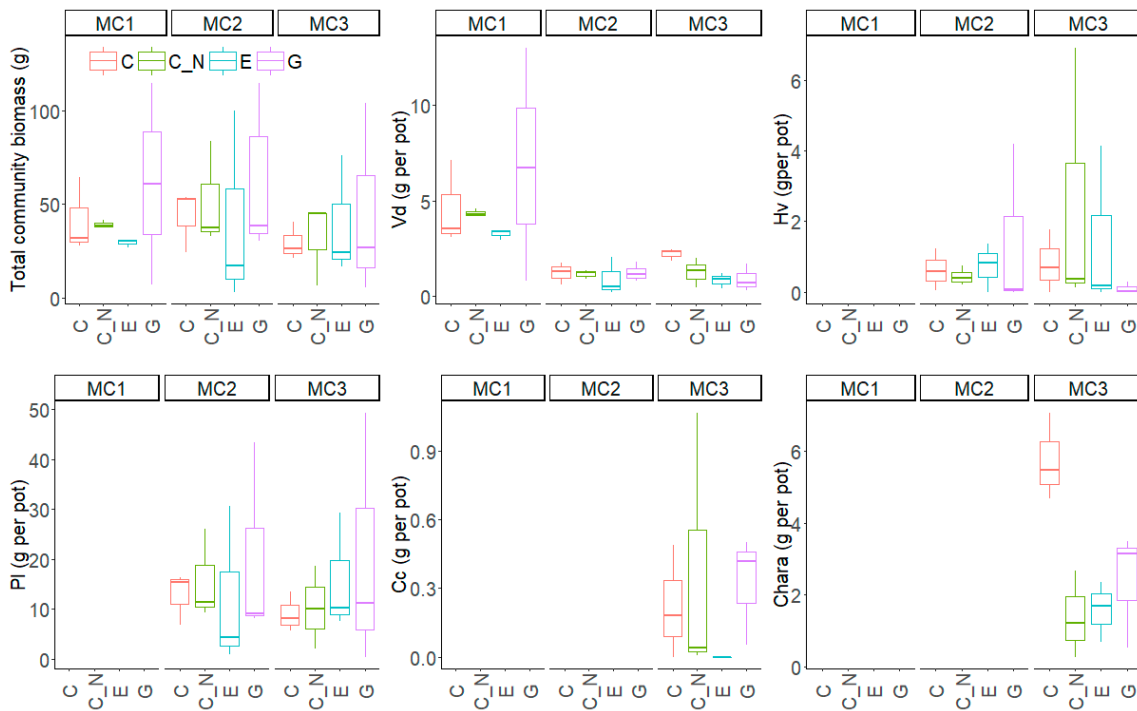
Figure 3. Total community biomass and biomass of five submerged macrophytes species (per pot) during the experiment for the three macrophyte community treatments (MC1, MC2 and MC3) and the four extreme precipitation treatments (EP: C, C_N, E and G). Vd = *Vallisneria denseserrulata*; Hv = *Hydrilla verticillata*; Pl = *Potamogeton lucens*; Cc = *Cabomba caroliniana*; Chara = *Chara sp.*
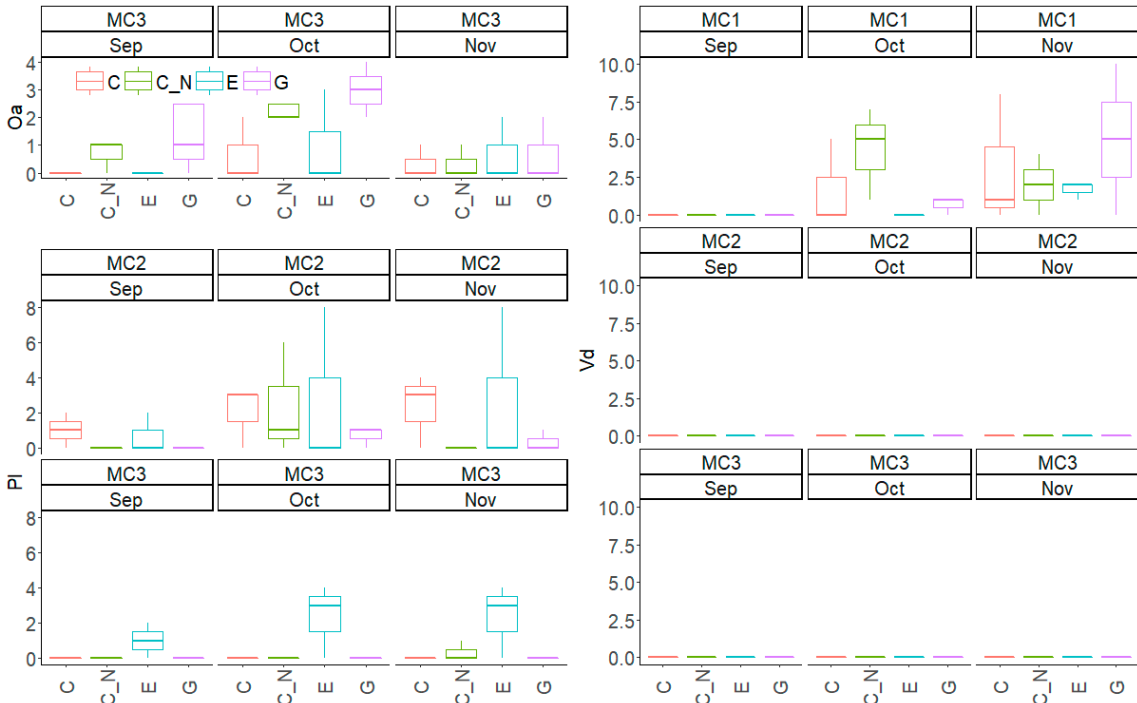
Figure 4. Number of flowering of three submerged macrophytes species in September (Sep), October (Oct) and November (Nov) in the experiment including three macrophyte community treatments (MC1, MC2 and MC3) and four extreme precipitation treatments (EP: C, C_N, E and G). Oa = *Ottelia alismoides*; Pl = *Potamogeton lucens*; Vd = *V. denseserrulata*.

The flowering of *O. alismoides* in the C_N and G treatment occurred earlier in September than in the other two treatments. The flowering number per tank did not differ between the four EP treatments (F = 2.09, $p > 0.05$), which were 0.7 ± 1.2 (C), 2.3 ± 1.6 (C_N), 1.0 ± 1.7 (E) and 3.0 ± 1.0 (G), respectively.

In contrast to *O. alismoides*, *P. lucens* had no flowers in C_N and G but flowered in C and E in September. It displayed delayed flowering in MC3 and had no flowers in October except in the mesocosms exposed to an extreme water level increase in E. Though no significant differences were found between the MC or EP treatments in the individual months (F < 2.95, $p > 0.05$ for MC and EP), *P. lucens* tended to have more flowers in E.

3.4. Biomass of Snails, Filamentous Algae and Epiphyton and Intactness Status of Leaves for V. denseserrulata and P. lucens

The biomass of filamentous algae on the concrete walls did not differ significantly among the treatments and averaged 25.3 ± 19.8 mg m^{-2} (Table 3, Figure 5). Snail biomass and abundance were similar and relatively large, averaging 15.9 ± 8.8 g m^{-2} and 99 ± 60 ind m^{-2}, respectively, but showing no significant differences among treatments.

Table 3. Statistical summary of two-way ANOVA on the effects of macrophyte community (MC: MC1, MC2 and MC3) and extreme precipitation (EP: C, C_N, E and G) on the biomass (or abundance) of filamentous algae, snails and epiphyton at the end of the experiment. NS = not significant; * = $p < 0.05$; *** = $p < 0.001$.

Indicators	Two-Way ANOVA		
	MC F Values, Sig.	EP F Values, Sig.	Interaction F Values, Sig.
Filamentous algae biomass	2,34, NS	0.72, NS	0.39, NS
Snail biomass	0.51, NS	0.72, NS	2.04, NS
Snail abundance	2.77, NS	1.07, NS	1.75, NS
Epiphyton on *V. denseserrulata*	1.93, NS	4.18, *	6.82, ***
Epiphyton on *P. lucens*	0.05, NS	0.88, NS	1.32, NS

The epiphyton biomass on *P. lucens* was 2.7 ± 2.4 g m^{-2} and thus significantly higher than that on *V. denseserrulata* (0.57 ± 0.77 g m^{-2}). The epiphyton biomass on *P. lucens* did not respond significantly to either the EP treatment, the MC treatment or their interaction, but the biomass on *V. denseserrulata* showed significant interaction between the EP and the MC treatment. After dividing the dataset into subsets for each MC treatment, epiphyton biomass on *V. denseserrulata* was significantly higher in C than in the other three EP treatments in MC1 (F = 14.71, $p < 0.001$), but no significant difference was found between the four EP treatments in either MC2 or MC3 (F < 3.01, $p > 0.05$). In addition, there was no significant correlation between snail biomass (or abundance) and the biomass of filamentous algae and epiphyton on the two submerged species ($p > 0.05$ for all correlation analyses).

The leaf intactness of the two species *P. lucens* and *V. denseserrulata* was evaluated based on division into three categories: intact, minor and major damages. The proportions of intact, minor or major damage of the total leaf numbers did not differ among the four EP treatments for either of the species (for all three types of leaf intactness, F < 2.52, $p > 0.05$). We also found that that the proportion of the intact *V. denseserrulata* leaves correlated negatively with snail abundance ($p < 0.05$; Figure S4), and the proportion of leaves with major damages positively with snail abundance ($p < 0.05$). A similar pattern was found for *P. lucens* (Figure S5). None of the proportions of the three categories of leaf intactness status correlated with snail biomass.
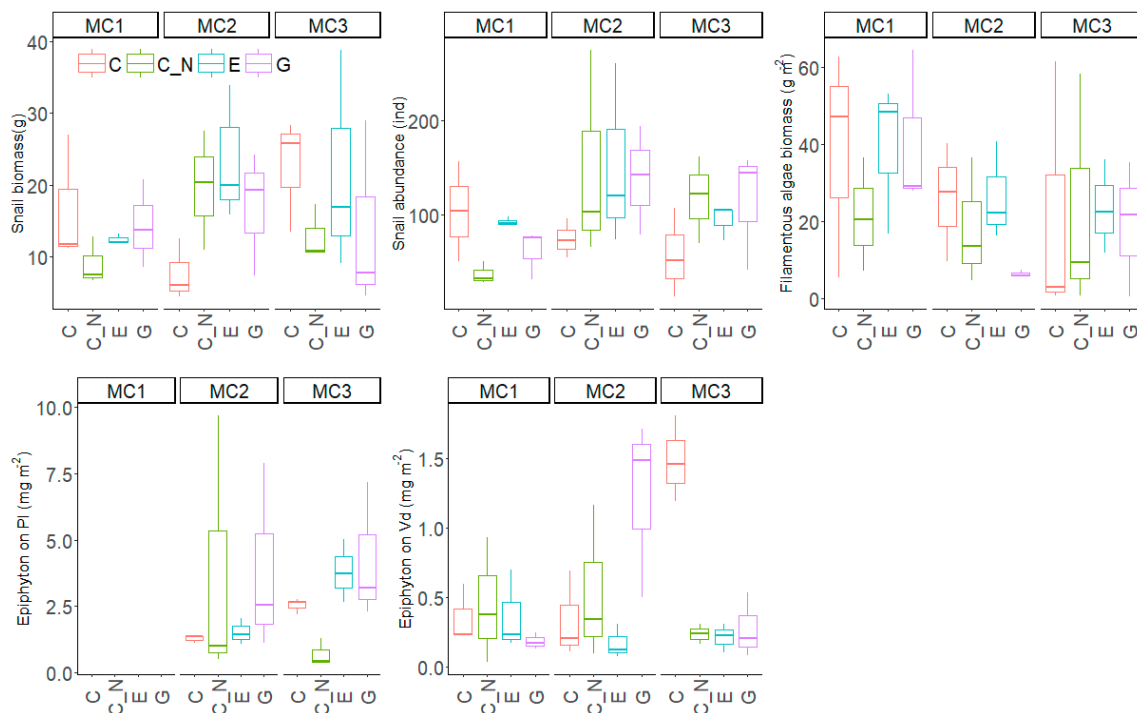
Figure 5. Snail biomass and abundance, biomass of filamentous algae and epiphyton on two submerged macrophytes at the end of the experiment for the three macrophyte community treatments (MC1, MC2 and MC3) and the four extreme precipitation treatments (EP: C, C_N, E and G). Pl = *Potamogeton lucens*; Vd = *V. denseserrulata*.

4. Discussion

As indicated by the biomasses of total macrophyte communities, snails, phytoplankton and two types of periphyton (filamentous algae and epiphyton on *P. lucens*), our freshwater ecosystems were resilient to a simulated extreme event and run under low phosphorus concentrations. Total biomasses did not differ among the three macrophyte communities, which supports our first hypothesis. However, phytoplankton Chl*a* was higher in the extreme water level increase treatment compared with the control for the macrophyte community consisting of *V. denseserrulata* only.

An epiphyton biomass of 10 mg m^{-2} has been reported to produce strong shading limiting the growth of submerged macrophytes [39]. A study involving experiments in glass tanks revealed that a snail biomass of approximately 10~20 g m^{-2} sufficed to reduce periphyton biomass to <5 mg m^{-2} and promote the growth of *V. spinulosa* [37]. In our study, snail biomass (15.9 ± 8.8 g m^{-2}) was comparable with that in [37] and, expectedly then, the grazing effect on epiphyton would be strong. Consistently, the epiphyton biomass on the two chosen species (*V. denseserrulata* and *P. lucens*) was low in all treatments, indicating high grazing from snails and thus probably low shading effects from epiphyton on the macrophytes. Previous studies revealed that flooding, resulting in a sudden increase of water level and a nutrient pulse, might strongly change the primary producers (e.g., biomass of benthic algae) in tropical river–floodplain ecosystems [40]. However, submerged macrophytes are known to have positive feedbacks maintaining a clear-water state in shallow lakes by outcompeting phytoplankton, reducing resuspension and removing excessive nutrients from the water column [8]. Although total nitrogen and nitrate were higher in the extreme treatment than in C, phytoplankton biomass and Kd were low in all treatments, probably due to phosphorus limitation of phytoplankton growth. The macrophytes included in our study can efficiently take up phosphorus from the sediment [41] and apparently did not suffer from nutrient and light limitation as the total macrophyte biomass was not affected by the simulated extreme precipitation.

Extreme events such as heatwaves have been reported to significantly affect the growth and reproduction of submerged macrophytes, and macrophytes have shown clear species-specific responses to extreme events [25,26]. In our study, species-specific responses to the different extreme precipitation treatments were also found depending on growth form. The height of slow-growing *Chara sp.* (approximately 40 cm) in the C treatment was twice as large as in other treatments, indicating that extra nutrient loading alone or together with a high water-level has adverse effects on the growth of charophytes [42,43]. Though well-known for its invasiveness [44], *C. caroliniana* had disappeared at the end of the experiment in the extreme treatment, perhaps due to high pH and low alkalinity inducing carbon limitation of the growth [24]. Both species were short-growing (<50 cm), so the shading from other higher macrophytes (e.g., *P. lucens*) might have been a contributing factor.

A significantly higher biomass of *V. denseserrulata* was found in the *V. denseserrulata*-only community than in the other two communities with diverse growth forms, which is consistent with the lower Kd in this treatment. A study conducted in the same mesocosms found contrasting responses of the leaf chlorophyll parameters of *V. denseserrulata* growing in different macrophyte communities, but the biomass and morphology of this species did not differ when growing in macrophyte communities with diverse growth forms [7]. The different results observed for the same species might be attributed to differences in pot size; the plastic cups (diameter top 6.8 cm, bottom 4.8 cm; height 74 cm) in [7] were small compared with the pots used in this study, which likely limited macrophyte growth and diminished the effects of the macrophyte community. Only for *P. lucens* did maximum plant height show significant responses to higher water levels, reflecting the growth form of this canopy-forming macrophyte type. On average, light intensity was reduced by 1/3 of surface radiation at 65 cm depth in the 150 cm water level treatments and at 50 cm depth in the 75 cm water level treatments. Accordingly, light availability may have been sufficient for growth of the species with large plant height, which may explain why the biomasses of *V. denseserrulata*, *P. lucens* and *H. verticillata* did not respond significantly to an increase in water level or nutrient loading. Significantly lower epiphyton biomass was found on *V. denseserrulata* than *P. lucens* leaves, probably indicating preference by snails for the epiphyton on this species. In addition, based on the leaf intactness, there is potentially direct grazing by snails on both species. Due to the low shading from epiphyton and the high proportion of intact and minor-damaged leaves, the direct effects of snails and epiphyton on macrophyte growth were assumed to be minor in our study compared with the studies in [37,45]. In summary, our results showed that the growth form combination of the macrophyte communities plays a more vital role in regulating the growth of rosette-like (or bottom-dwelling) species than of canopy-forming species, which supports our second hypothesis. Moreover, the short species (*Chara sp.*) was more affected by extreme precipitation than the taller species (*V. denseserrulata*), even though they have a similar growth form.

The sexual reproduction of the three submerged macrophytes also showed species-specific responses. Generally, *O. alismoides* tolerates moderate water level fluctuations, but sexual reproduction exhibited a decreasing trend at the water level change of 75 cm [28]. Flower numbers did not differ among the four extreme precipitation treatments (even for the 75 cm increase), but the flowering phase advanced after nutrient addition alone or a gradual water level increase. With the pollination mode of entomophily [46], the change in the flowering phase of *O. alismoides* might affect interaction with pollinators. *O. alismoides* is an annual species and did not recover after winter in the experiment, preventing us from evaluating the effects of extreme precipitation on biomass at the end of the experiment. The responses of flowering also depend on growth form; thus, the rosette-type macrophyte *V. denseserrulata* produces female flowers at the bottom of the plant, while the canopy-forming *P. lucens* flowers at the growing tips after the tips reach the water surface [30]. Thus, *P. lucens* elongated to the surface even in the extreme treatment and tended to exhibit advanced florescence with more flowers, while *V. denseserrulata* likely faced a higher cost of producing female flowers in deeper water [47].

5. Conclusions

In conclusion, we found that, at community level, high-coverage macrophytes were resilient to one event of extreme precipitation and at species level, species-specific responses were found for macrophyte biomass and sexual reproduction. We conclude that the growth form of submerged macrophytes is important when considering the effects of climate change and that maximum plant height is an important trait in the adaptation of submerged macrophytes to extreme precipitation. It should be noted that our results were obtained at low phosphorus concentrations and cannot readily be transferred to more nutrient-rich conditions.

Conflicts of Interest: The authors declare no conflict of interest.

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