



Indirect effects of extreme precipitation on the growth of *Vallisneria denseserrulata* Makino

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ABSTRACT

Submerged macrophytes are important for maintaining a clear water state in shallow lakes but are now threatened by the ongoing climate changes involving more extreme weather events. We explored the effects of extreme precipitation (higher water level, higher loading of nutrients (phosphorus and nitrogen)) on the growth of the submerged macrophyte *Vallisneria denseserrulata* Makino in a 10-month long mesocosm experiment. We used three macrophyte communities (one, three and six species) and four simulated scenarios of water level and nutrient loading (constant 75 cm, no nutrient loading; gradual water level increase from 75 cm to 150 cm over three months; extreme water level increase to 150 cm within one day at the same nutrient loading as in the gradual increase; constant 75 cm and the same nutrient loading as in the two former treatments). Leaf chlorophyll fluorescence, biomass, morphology of macrophytes and periphyton biomass were recorded. In the treatment with an extreme increase in water level, macrophyte biomass declined, whereas plant height and leaf number remained unchanged compared with the treatments where only nutrient but not water levels were increased. The combined effect of increased water level and enhanced nutrient loading (gradual and extreme change) did not differ significantly from the constant 75cm treatment without additional nutrient loading. No significant differences were found in epiphyton biomass among the water level treatments, while epiphyton biomass was marginally higher and plant volume inhabited lower in the two treatments with a water level increase. Changes in macrophyte assemblages only had limited effects on maximum leaf width and the relative electron transport rate of *V. denseserrulata*. In conclusion, *V. denseserrulata* was overall resilient to the major simulated changes in precipitation, except when the water level increased suddenly, and only marginally influenced (leaf chlorophyll fluorescence parameter) by the composition of the macrophyte assemblage.

1. Introduction

The Intergovernmental Panel on Climate Change (IPCC) has predicted that the frequency of extreme climate events (heavy rainfall, droughts and heat waves) will increase in the future (Field et al., 2014), which, even if they are of short-term duration, may have significant effects on the structure and function of ecosystems, including lakes (Lesack et al., 2013; Neif et al., 2017; Audet et al., 2017). For example, changes in precipitation may affect nutrient concentrations and induce a sudden shift in the structure and function of temperate lake ecosystems (Coops et al., 2003; Alberta et al., 2014). Extreme precipitation may lead to a sudden water level increase and pulses of nutrient input, which potentially will affect the distribution patterns and growth of submerged macrophytes (Hidding et al., 2014; Zhang et al., 2014).

Previous studies of the effects of extreme precipitation on submerged macrophytes have focused on either the effects of water level fluctuations (Evtimova and Donohue, 2016; Wang et al., 2016) or pulse nutrient loading (Zhang et al., 2016; Olsen et al., 2017). Major water level fluctuations (± 75 cm) have been revealed to adversely affect the growth of aquatic plants (Deegan et al., 2007; Yu and Yu, 2009; Zhang et al., 2012). In contrast, pulses of nitrogen had no or only temporary effects on the biomass accumulation of submerged macrophytes under limited phosphorus conditions (Zhang et al., 2016; Olsen et al., 2017). Extreme precipitation causes, however, both pulsed nutrient inputs and sudden increases in water level with subsequent effects on submerged macrophytes, rendering precipitation an important element to be considered besides nutrient pulses and water level alterations.

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Shallow lakes with low nutrient levels are commonly dominated by submerged macrophytes (Moss et al., 2013) with strong positive feedback mechanisms that help maintain the clear water state (Scheffer et al., 1993). In temperate lakes, submerged macrophytes have been shown to play an important structural role by, for instance, providing a refuge for zooplankton allowing them to escape fish predation and by reducing sediment suspension induced by wind or benthic fish (Jeppesen et al., 1998; Li et al., 2008). In addition, submerged macrophytes also interact with periphyton on submerged macrophytes (epiphyton) and on sediment (epipelon) (Cano et al., 2013; Cao et al., 2014, 2017). Submerged macrophytes can be divided into several different growth forms and classified into two types: slow-growing rosette (e.g. vallisnerid *Vallisneria* spp.) and canopy-forming (e.g. magnopotamid *Potamogeton lucens*) species (Vis et al., 2006; Kelly et al., 2015). The slow-growing type allocates most of its biomass to leaves and stems near the lake bottom, while the canopy-forming type allocates a greater proportion of biomass to leaves and stems near the water surface. Thus, different micro-habitats with distinct structures are created within the macrophyte beds (affected by, for instance, light attenuation conditions) (Madsen et al., 1993). These distinct structures may influence the interaction with other macrophytes, periphyton and phytoplankton (Vis et al., 2006; Kelly et al., 2015; Hao et al., 2017). Therefore, the effects of extreme precipitation may vary relative to the different macrophyte growth forms and the combinations in which they occur.

In this study, we investigated the indirect effects of extreme precipitation on the growth of the submerged macrophyte *V. denseserrulata* in clear water mesocosms over a 10-month period. Three different types of macrophyte communities were constructed using *V. denseserrulata* and five other species. Two extreme precipitation effects (i.e. changes in water level and nutrient loading) were in focus. We hypothesised that: 1) at the same level of nutrient loading, a rapidly increasing water level would strongly reduce the growth of *V. denseserrulata* compared with a gradual increase or absence of an increase in water level. Moreover, pulsed input of nutrients would lead to decreased macrophyte growth due to the extensive shading induced by the increase in phytoplankton and epiphyton biomass. 2) The growth response of *V. denseserrulata* would differ physiologically and/or morphologically depending on the combination of plants – mixed growth with other vallisnerid species or with magnopotamid species such as *P. lucens* – as different structures of dominant species create contrasting micro-habitats (e.g. different interception of light).

2. Material and methods

2.1. Experimental material and design

The slow-growing *V. denseserrulata* is a perennial submerged macrophyte species. It maintains an above-ground biomass in winter and is found mainly in central-southern China. The macrophytes used in the current experiment were collected in small ponds in Wuhan Botanical Garden, China. One individual of similar size (length ca. 10 cm) was planted into plastic cups (top diameter x bottom diameter x height: 68 mm x 48 mm x 74 mm) filled two-thirds with soil pre-soaked in lake water for one week. Six cups were placed in each mesocosm in a greenhouse. According to the experimental design, 36 concrete mesocosms (four water level scenarios and three macrophyte communities, each treatment with 3 replicates) (length x width x depth: 1 m x 1 m x 1.5 m) were used in the experiment, comprising a total of 216 cups.

Treatment 1: Water level

There were four water level treatments, ambient (with or without nutrient loading), extreme and normal precipitation. In the ambient treatment (CK), the water level was kept stable at 75 cm throughout the experiment. In the extreme treatment (E), a sudden increase in water level to 150 cm within one day was created to simulate an extreme precipitation event. In the “normal” treatment (G), the water level was gradually increased to 150 cm over a 3-month period (25 cm monthly).

To investigate the effects of extreme precipitation together with nutrient loading, we designed a treatment (CK_N) with stable water level (75 cm) but with additional nutrient loading. Tap water (total nitrogen: $2 \pm 0.23 \text{ mg L}^{-1}$, total phosphorus $60 \pm 8 \mu\text{g L}^{-1}$, N:P molar ratio ca. 74:1) was used to simulate the increase in water level. The nutrient increase was achieved by addition of chemicals (KNO_3 and KH_2PO_4). E, G and CK_N were manipulated to ensure similar loadings of nitrogen and phosphorus based on mass balance calculations. The water loss was very small (1–2 cm per month) during the experiment, and tap water was used to maintain the designed water level. Previous studies in the same mesocosm system have shown the effects of the water addition needed to top-up the lost water had negligible effect on the nutrient loading to the system (E.H. Li et al., 2008; W. Li et al., 2008; Zhang et al., 2016).

Treatment 2: Macrophyte communities

Three different macrophyte communities were created to represent different community structures (hereafter referred to as C1, C3 and C6). Five other aquatic plant species were used, including *Potamogeton lucens* L., *Hydrilla verticillata* L., *Cabomba caroliniana* Gray., *Ottelia alismoides* Pers. and *Chara* sp., collected from Wuhan Botanical Garden, China, where they grow naturally in small ponds. *Vallisneria denseserrulata*, *P. lucens* and *H. verticillata* are three of many commonly found submerged species in natural lakes and were selected due to their different growth forms. The remaining three species were chosen randomly from the many candidates naturally occurring in lakes. C1 held *V. denseserrulata* (Vd) only. In this treatment, nine pots (top diameter 27 cm, bottom diameter 22 cm, height 14 cm, two-thirds filled with soaked soil) were placed in one mesocosm, each pot containing two Vd individuals. C3 included three species, *V. denseserrulata* (Vd), *P. lucens* (Pl) and *H. verticillata* (Hv). Three pots of each species were randomly placed in one mesocosm, the pots containing, respectively, four Hv, three Pl and two Vd individuals to obtain a roughly similar initial biomass in each pot. C6 included the same three species as C3 and additionally three relatively rare species: *C. caroliniana* (Ca), *Chara* sp. (Ch) and *O. alismoides* (Oa). Two pots of each of the first three species and one pot of each of the three rare species were placed in each mesocosm, i.e. 9 pots in total. As to the rare species, three Ca and Oa individuals and a small clump of Ch were used, ensuring a total biomass of 1.5 g fresh weight in all pots to obtain similar initial conditions in all mesocosms.

The experiment lasted from August 2015 to May 2016. Macrophyte, epiphyton and water samples were collected in August (Aug), September (Sep), October (Oct), November (Nov), January (Jan) and May (May), and the water level increase in G and E was initiated in September. On each sampling event, the water samples represented the entire water column and were taken with a tube sampler and thoroughly mixed before analysis. Concentrations of total nitrogen (TN) and total phosphorus (TP) were recorded using spectrophotometric methods after digestion with $\text{K}_2\text{S}_2\text{O}_8$ solution, and water chlorophyll a (Chla) was determined by filtering 1 l of water on a Whatman GF/C filter after ethanol extraction (Huang et al., 1999). For macrophyte and epiphyton samples, we randomly chose one cup and removed the second mature leaf of the macrophytes to determine the relative electron transport rate (ETR) using PAM 2100 (Jiang et al., 2017). Afterwards, the leaf was stored in a plastic bag at 4 °C for determination of epiphyton biomass in the lab (Cao et al., 2015). The remaining macrophytes were harvested and cleaned in the lab, together with the selected leaves, for determination of leaf morphology (maximum length and width) and dried at 80 °C for 48 h to calculate (above- and below-ground) biomass. Since *Vallisneria* is a genus with clonal individuals, ramet number, i.e. the number of the clonal individuals, was also recorded. The ratio of below-ground and above-ground biomass (BG/AG) was calculated. Epipelon was sampled applying a plastic syringe and extracted by ethanol for Chla analysis (Cao et al., 2017; Jiang et al., 2017). Plant volume inhabited (PVI) of the total macrophyte community in the mesocosms was recorded monthly from Jan 2016 to May 2016.

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