



Consecutive submergence and de-submergence both impede growth of a riparian plant during water level fluctuations with different frequencies

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ABSTRACT

Repeated exposure to submergence and de-submergence may induce acclimation in plants growing in riparian areas. However, the effect of each consecutive submergence and de-submergence event has not been evaluated separately. We subjected a riparian species *Alternanthera philoxeroides* to two different fluctuation frequencies: low fluctuation frequency (LFF) and high fluctuation frequency (HFF). Consecutive submergence and de-submergence had comparable negative effects on growth of *A. philoxeroides*, while they respectively down- and up-regulated photosynthetic electron transport in both LFF and HFF. The submergence effects on growth were significantly smaller in the 2nd cycle than in the 1st cycle of LFF, suggesting reduced tissue loss in the 2nd cycle as a result of acclimation. In HFF, the growth of *A. philoxeroides* was more strongly suppressed than in LFF. During de-submergence, biomass increased in both control and de-submerged plants in LFF, whereas growth recovery was not always seen in HFF. At the end of the experiment, the treatment plants in HFF had only ~50% biomass of the corresponding plants in LFF. Although HFF enhances tissue loss during submergence and thus impairs growth recovery more strongly during de-submergence than LFF, both LFF and HFF induced photosynthetic, photoprotective or growth acclimation in *A. philoxeroides*.

1. Introduction

Water level fluctuations are major events that influence riparian and littoral ecosystems along streams and lakes (McGowan et al., 2011; Hirabayashi et al., 2013; Garssen et al., 2015). During water level fluctuations, by which riparian plants are subjected to frequent submergence and de-submergence, light transmission and gas diffusion change greatly, leading to extreme variations in availability of light, O₂ and CO₂ for photosynthesis and respiration of these plants (Colmer et al., 2013; Voeselek et al., 2016; Sasidharan et al., 2018). Furthermore, submergence and de-submergence also strongly influence redox potential and concentrations of nutrients and toxic compounds in riparian soil (Leyer, 2005; Colmer et al., 2013; Baastrup-Spohr et al., 2016). The toxic compounds include potentially toxic ions such as Mn²⁺ and Fe²⁺, and some volatile organic acids such as propionic and butyric acids, which can accumulate in water-saturated soil and damage roots (Greenway et al., 2006). Therefore, water level fluctuations have profound effects on plant performance and community assembly (Garssen et al., 2015; Baastrup-Spohr et al., 2016).

Fluctuation frequency (i.e., the number of cycles of water level changes within a certain time period) is one of the most important factors of water level fluctuations (Nilsson and Svedmark, 2002; Gerard et al., 2008; De Jager, 2012). Global climate change is predicted to increase the frequency of intense precipitation events in most temperate regions (IPCC, 2014). Consequently, hydrological interaction between rivers and surrounding riparian regions may be altered significantly, resulting in more frequent water fluctuation events in the near future (Hirabayashi et al., 2013; Garssen et al., 2015).

Increasing fluctuation frequency can become very challenging due to frequent variations in O₂ and light (Bornette et al., 2008; Baastrup-Spohr et al., 2016). Frequent changes in O₂ level leads to production of harmful reactive oxygen species and acetaldehyde in plant tissues (Blokina et al., 2003; Boamfa et al., 2005; Sasidharan et al., 2018), and frequent variations in light intensity may damage the photosynthetic apparatus (Luo et al., 2009; Voeselek et al., 2016). Water fluctuations with high frequency disturb seedling establishment, damage tissues and impair growth and reproduction of sensitive species (Casanova and Brock, 2000; De Jager, 2012; Baastrup-Spohr et al., 2016). However,

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low or intermediate frequency of water level fluctuations has little effect on, or even promote establishment and growth of some species (Casanova and Brock, 2000; Leyer, 2005; Cunha et al., 2006; Stokes et al., 2010).

In fact, riparian species differ in their responses to frequent water level fluctuations (Casanova and Brock, 2000; Gerard et al., 2008; Luo et al., 2016; Striker et al., 2017). Tolerant species can quickly resume photosynthetic capacities and/or establish gas diffusion between submerged and emerged tissues to alleviate energy and O₂ deficiency in roots and/or rhizomes, while sensitive species cannot. Such differences in their response may be related to their habitats along riparian regions to which they are adapted or acclimated (Nakai and Kisanuki, 2011; Baastrup-Spohr et al., 2016; Winkel et al., 2016). For example, plants at higher elevations may experience water level fluctuations less frequently than those at lower elevations (Gerard et al., 2008; McGowan et al., 2011; De Jager, 2012). In general, published reports are at variance concerning the effects of fluctuation frequency on plant growth (Casanova and Brock, 2000; Leyer, 2005; Stokes et al., 2010; McGowan et al., 2011; Baastrup-Spohr et al., 2016).

Acclimation to consecutive fluctuation cycles is of central importance for survival and growth of plants during water level fluctuations (Dylewski et al., 2012; Milroy and Bange, 2013). Many studies have investigated growth and photosynthetic responses of riparian plants to water level fluctuations, focusing on the overall performance at the end of fluctuations (Casanova and Brock, 2000; Nakai and Kisanuki, 2011; Dylewski et al., 2012; Milroy and Bange, 2013; Striker et al., 2017). In comparison, dynamic responses to consecutive fluctuation cycles have not been examined extensively (Nakai and Kisanuki, 2011; Milroy and Bange, 2013). Importantly, the effect of each submergence and de-submergence event, separated from the effect of the preceding cycle(s), has not been assessed (Baastrup-Spohr et al., 2016; Dolinar et al., 2016; Striker and Colmer, 2017).

Here we investigated the effects of fluctuation frequency in *Alternanthera philoxeroides* (alligator weed) which typically inhabits shallow water where it naturally experiences water level fluctuations (Zhang et al., 2015). Following a single submergence event, this species is able to quickly recover the photosynthetic capacity (Luo et al., 2009). We subjected the plants to water level fluctuations with different frequencies: low-frequency fluctuation (LFF), high-frequency fluctuation (HFF) and the corresponding control treatments to assess the impact of each submergence and de-submergence cycle. Specifically, we tested the following three hypotheses: (1) consecutive submergence or de-submergence events have negative effects on growth of *A. philoxeroides*, but (2) negative effects of later cycles may become smaller if acclimation helps the plant to better cope with the fluctuations, and (3) HFF may induce acclimation in *A. philoxeroides* more effectively than LFF as this species is adapted to shallow water conditions.

2. Material and methods

2.1. Plant species

Alternanthera philoxeroides (Mart.) Griseb. (Amaranthaceae) can survive in aquatic, semi-aquatic and terrestrial environments and is found frequently in riparian regions (Zhang et al., 2015). This species is native to South America but highly invasive in many countries including China. It can spread vegetatively and produces hollow, creeping stolons at the water surface. When completely submerged, it quickly elongates shoots to regain the contact with the atmosphere (Luo et al., 2009).

2.2. Material preparations

Plants of *A. philoxeroides* were collected from five clumps, at least 10 m apart from each other, of two wetlands in Taizhou, Zhejiang Province, China. The genetic variation of this species is very low in

China and plants from different clumps are probably from the same genet (Xu et al., 2003). Therefore, the collected plants were mixed and propagated vegetatively for two months in a greenhouse of Beijing SFK Technology Co., Ltd. Stolon fragments, each 15 cm long with five nodes and an apex, were obtained from the stock population and planted in pots (17 cm in diameter × 14 cm in height) filled with 1:1 (v:v) mixture of peat and sand containing $0.65 \pm 0.05 \text{ g kg}^{-1}$ total phosphorus, $0.48 \pm 0.03 \text{ g kg}^{-1}$ total nitrogen and $7.83 \pm 0.62 \text{ g kg}^{-1}$ total organic carbon (mean ± s.e., n = 4).

2.3. Experimental design

After two weeks from planting, 192 plants of similar size were selected for the experiment. Eight of them were harvested to measure initial plant height and biomass: $30.71 \pm 1.16 \text{ cm}$ and $1.04 \pm 0.08 \text{ g}$ (mean ± s.e.), respectively. The remaining 184 plants were randomly assigned to three treatments: (1) control, (2) LFF and (3) HFF. The control plants were kept in waterlogged conditions, as were the plants during de-submergence treatment. In LFF, the water level for the plants changed from 0 to 150 cm deep (submergence) and then back to 0 cm (de-submergence), which was repeated twice (two cycles of ten-day submergence followed by ten-day de-submergence) during a 40-day experiment from 5 July to 13 August 2014 (Fig. 1). Also in HFF the water level fluctuated between 0 and 150 cm, but each cycle lasted only ten days (i.e., five-day submergence followed by five-day de-submergence), resulting in four cycles during the same experimental period. Notably, the control plants of the 1st submergence treatment in LFF and the control plants of the 1st de-submergence treatment in HFF

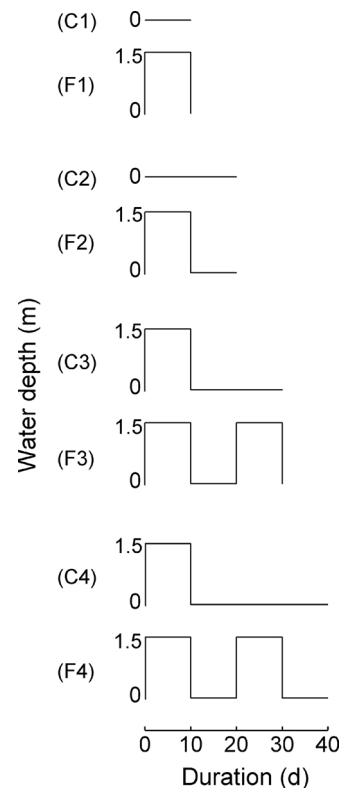


Fig. 1. Schematic representation of eight treatments of low-frequency water level fluctuations (LFF), consisting of four fluctuation treatments (F1–F4) and four corresponding control treatments (C1–C4). The four fluctuation treatments included two submergence (F1 and F3) and two de-submergence (F2 and F4) events, lasting ten days for each event. Similarly, high-frequency fluctuations (HFF) had eight fluctuation and eight corresponding control treatments during the same 40-day period. The eight fluctuation treatments included four submergence and four de-submergence events, lasting five days for each event.

were all waterlogged for ten days, from day 1 to day 10. Therefore, we kept only one control treatment for these two treatments.

To separate the effects of each submergence/de-submergence cycle, the control plants were defined as those that experienced LFF or HFF until the one cycle to be evaluated. For the 1st submergence treatment in LFF, the control plants were kept waterlogged for ten days (Fig. 1). For the 1st de-submergence treatment, the control plants were waterlogged for another ten days (in total 20 days). For the control of the 2nd submergence and de-submergence treatment, the plants that experienced the 1st submergence/de-submergence cycle were brought to the waterlogged condition for 10 or 20 days, respectively (Fig. 1). The definition of the control treatment was also the same in HFF. The plants survived in all treatments.

The experiment was conducted by using eight black plastic tanks (150 cm in diameter and 165 cm in height) filled with tap water and placed outdoor at the botanical garden of Beijing SFK Technology Co., Ltd. The pots were suspended in the tanks by using ropes that were tied to a steel frame mounted on top of each tank. Each tank contained 23 pots (plants). Water level fluctuation was applied by changing the vertical position of pots in the tank; releasing the rope increased the flooding depth whereas pulling up the rope decreased the flooding depth. The treatment plants were completely submerged under 150-cm-deep water during submergence events and were waterlogged during de-submergence events; however, two of them grew out of the water from day 30 and were thus only partially submerged during the subsequent time of water level fluctuations. Eight replicate plants from eight tanks (one plant per tank) were analyzed for each treatment (submergence or de-submergence and the corresponding control) at each time point.

2.4. Meteorological parameters of experimental site

Data on daily solar radiation, mean/maximal/minimal temperature, relative air humidity and precipitation during the experimental period were obtained from the publicly available Chinese Meteorological Data Sharing Service System (<http://data.cma.cn/>). These data were collected at the weather station which was nearest from the botanical garden of Beijing SFK Technology Co., Ltd. between 5. July and 13. August 2014. The average daily solar radiation, maximum air temperature, relative air humidity and the sum of daily precipitation for each five-day period during the experiment ranged between 12–26 MJ m⁻², 32–35 °C, 44–75% and 0–42 mm, respectively (Supplementary Table S1). Photosynthetically active radiation measured by a Li-250 A photometer (Li-Cor Biosciences, Lincoln, NE, USA) at water surface at noon was 800–1800 μmol photons m⁻² s⁻¹. To maintain the water level in tanks, tap water was added to compensate for evaporation loss while surplus water was removed after rain.

2.5. Physico-chemical properties of floodwater

The temperature, pH and dissolved O₂ content of floodwater were measured with a YSI Quatro multi-parameter water quality meter (Fondriest Environmental, Fairborn, OH, USA) in the morning (10:00–12:00) at the beginning (day 0) and at the end of treatments (day 40). For measurements, the sensor of the instrument was placed 50 cm below the water surface of each flooding tank. The pH and dissolved O₂ of floodwater increased from day 0 to day 40 at similar water temperatures (Table S2).

2.6. Growth analyses

After each submergence or de-submergence, plants were harvested and divided into adventitious roots, shoots and roots. The plant materials were then dried at 80 °C for 72 h and weighed.

2.7. Chlorophyll fluorescence measurements

The maximal quantum yield of photosystem II (Fv/Fm) was determined by chlorophyll fluorescence measurements. Measurements were conducted on the youngest fully-expanded leaves (i.e., the mature leaves closest to the growth zone of the shoot) by using a pulse modulated fluorometer (PAM-2500, Heinz Walz, Germany) at 09:00–12:00 h local time. The maximum and minimum fluorescence in dark-adapted leaves (Fm and Fo, respectively) were measured after 30 min of dark adaptation using leaf clips. The intensity and duration of the saturation pulse applied to determine Fm were 3500 μmol photons m⁻² s⁻¹ and 1 s, respectively. Fv/Fm was calculated as Fv/Fm = (Fm - Fo)/Fm (Maxwell and Johnson, 2000). The leaves were then exposed to white actinic light of 800 μmol photons m⁻² s⁻¹ for 4 min prior to the measurement of steady-state fluorescence (Fs) and the maximal fluorescence in light-adapted leaves (Fm'). The effective quantum yield of photosystem II in the light (ΔF/Fm') was calculated as ΔF/Fm' = (Fm' - Fs)/Fm', and non-photochemical energy quenching (NPQ) in photosystem II as NPQ = (Fm - Fm')/Fm' (Maxwell and Johnson, 2000).

2.8. Data analyses and statistics

Data on daily solar radiation, daily mean/maximal/minimal temperature and daily relative air humidity were averaged, and data on precipitation were summed up for every five days during the experimental period.

Effects of each submergence/de-submergence cycle were assessed for growth and photosynthetic parameters. The submergence effects of the 1st fluctuation cycle were calculated as the difference between the submerged plants and the corresponding control plants at the end of the 1st submergence (F1 - C1 in Fig. 1 for LFF), and the de-submergence effects as the incremental difference that developed between the de-submerged plants and the corresponding control plants during the 1st de-submergence (i.e., (F2 - C2) - (F1 - C1)). Similarly, the submergence effects of the 2nd fluctuation cycle were calculated as F3 - C3 and the de-submergence effects as (F4 - C4) - (F3 - C3). Following this principle, submergence and de-submergence effects were also calculated for HFF. The differences between the treatments were evaluated for each tank separately, resulting in eight replicate values at each time point.

We used one-sample *t*-test to check whether the effects of each submergence or de-submergence differed significantly from zero (no effect). One-way ANOVA followed by Duncan test was used to test whether the effects of submergence or de-submergence differed among consecutive fluctuation cycles in LFF or HFF. The differences of growth and photosynthetic characteristics were compared among treatments by Duncan test on day 20 and day 40; on these days the plants in LFF and HFF had the same total duration of submergence and de-submergence. Before the analyses, all data were checked for homogeneity of variance. Effects were considered to be significant if *p* < 0.05. All analyses were performed by using SPSS 16.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. Plant growth in LFF and HFF

All plants of *A. philoxeroides* survived the 40-day water level fluctuations in LFF and HFF, demonstrating that this species is well-adapted to water fluctuations. Fig. 2 shows the changes in plant dry weight in the LFF and HFF treatments: total mass, shoot mass, belowground root (BGR) mass, adventitious root (AR) mass and root-to-shoot (R/S) ratio. As the control and the treatment plants of the 2nd fluctuation cycle have experienced the 1st fluctuation cycle, the symbols of the 2nd cycle plants (both control and treatment) were connected to the de-submerged plants of the 1st cycle (Fig. 2). The symbols were connected in the same way also for the 3rd and the 4th cycles of HFF.

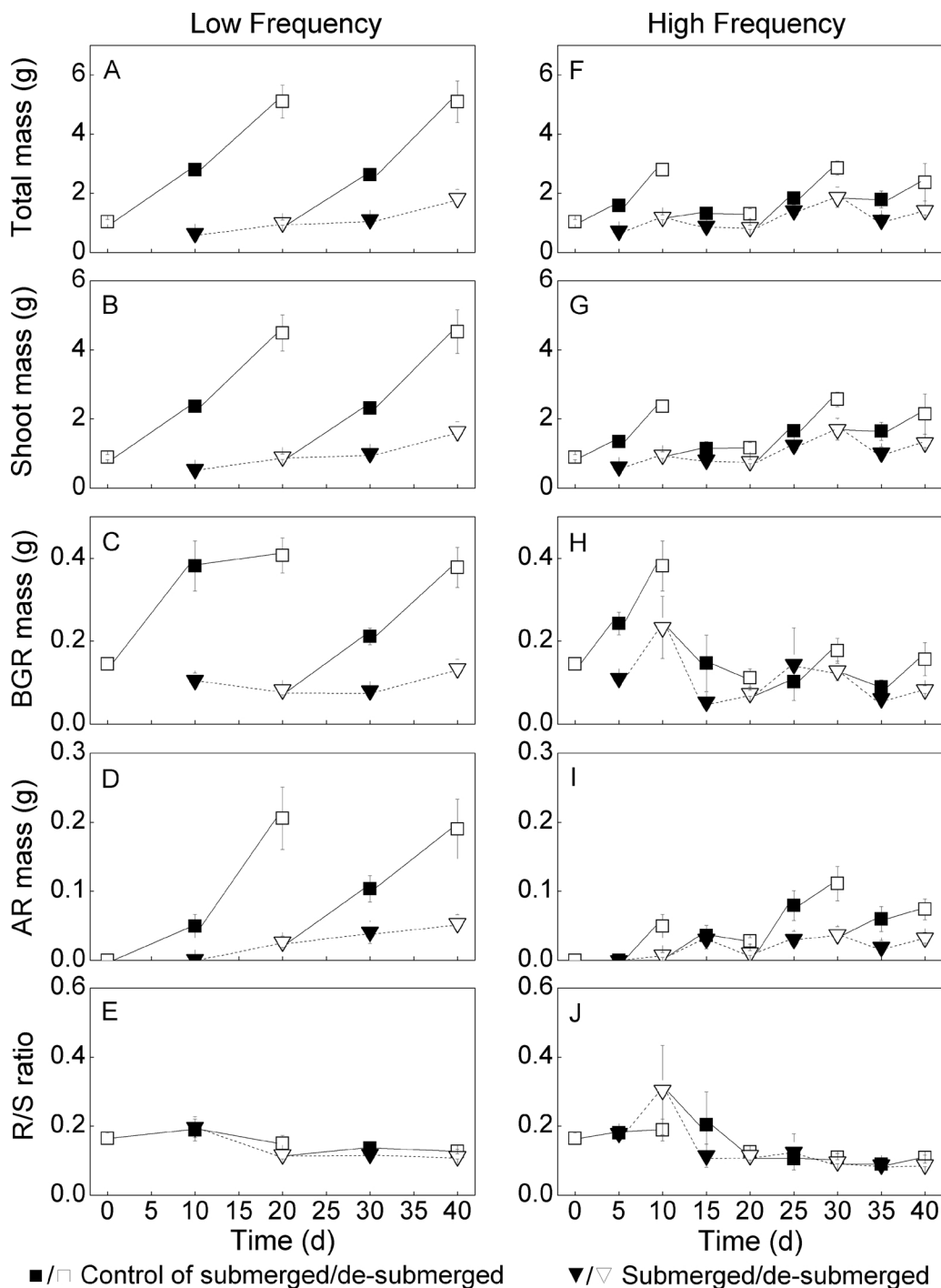


Fig. 2. Changes in total mass (A and F), shoot mass (B and G), belowground root mass (BGR mass, C and H), adventitious root mass (AR mass, D and I) and root to shoot ratio (R/S ratio, E and J) of *Alternanthera philoxeroides* during low- (A–E) and high-frequency (F–J) water level fluctuations. As the plants for the 2nd cycle of low-frequency fluctuation (C3 and F3 in Fig. 1) have experienced the 1st cycle, the symbols of the 1st de-submerged plants (F2) were connected to the symbols of the control and submerged plants of the 2nd cycle (C3 and F3). The symbols were connected in the same way for the 3rd and 4th cycles of high-frequency fluctuation. Symbols are mean values (\pm s.e., $n = 8$).

The water level fluctuations strongly inhibited growth of *A. philoxeroides* during submergence and de-submergence in both LFF and HFF (Fig. 2). At the end of the experiment on day 40, the plants, which went through 2×20 -day cycles in LFF, had nearly twice as much dry weight as the plants which experienced 4×10 -day cycles in HFF (Fig. 2A–D, F–I). However, R/S ratio was not significantly affected by the treatments, staying mostly between 0.1 and 0.2 (Fig. 2E and J). In LFF, the differences in dry weight between the treatment plants and the corresponding control plants were much larger at the end of the de-submergence treatment on day 20 and day 40 than after the preceding submergence treatment on day 10 and day 30 (Fig. 2A–D). The only exception was BGR mass which did not increase in the control plants between day 10 and day 20 in the 1st cycle (Fig. 2C). Increased dry

mass accumulation in de-submerged plants was also observed in HFF, namely, between day 5 and day 10 in the 1st cycle and between day 25 and day 30 in the 3rd cycle (Fig. 2). This was not the case in the 2nd (between day 15 and day 20) and the 4th (day 35 and day 40) cycles due to weak growth of both control and treatment plants in these periods.

3.2. Relative effects of each submergence/de-submergence cycle on plant growth

Each of the consecutive submergence and de-submergence events had negative effects on the growth of *A. philoxeroides* in both LFF and HFF (Fig. 3). The negative effects of ten-day submergence (closed bars)

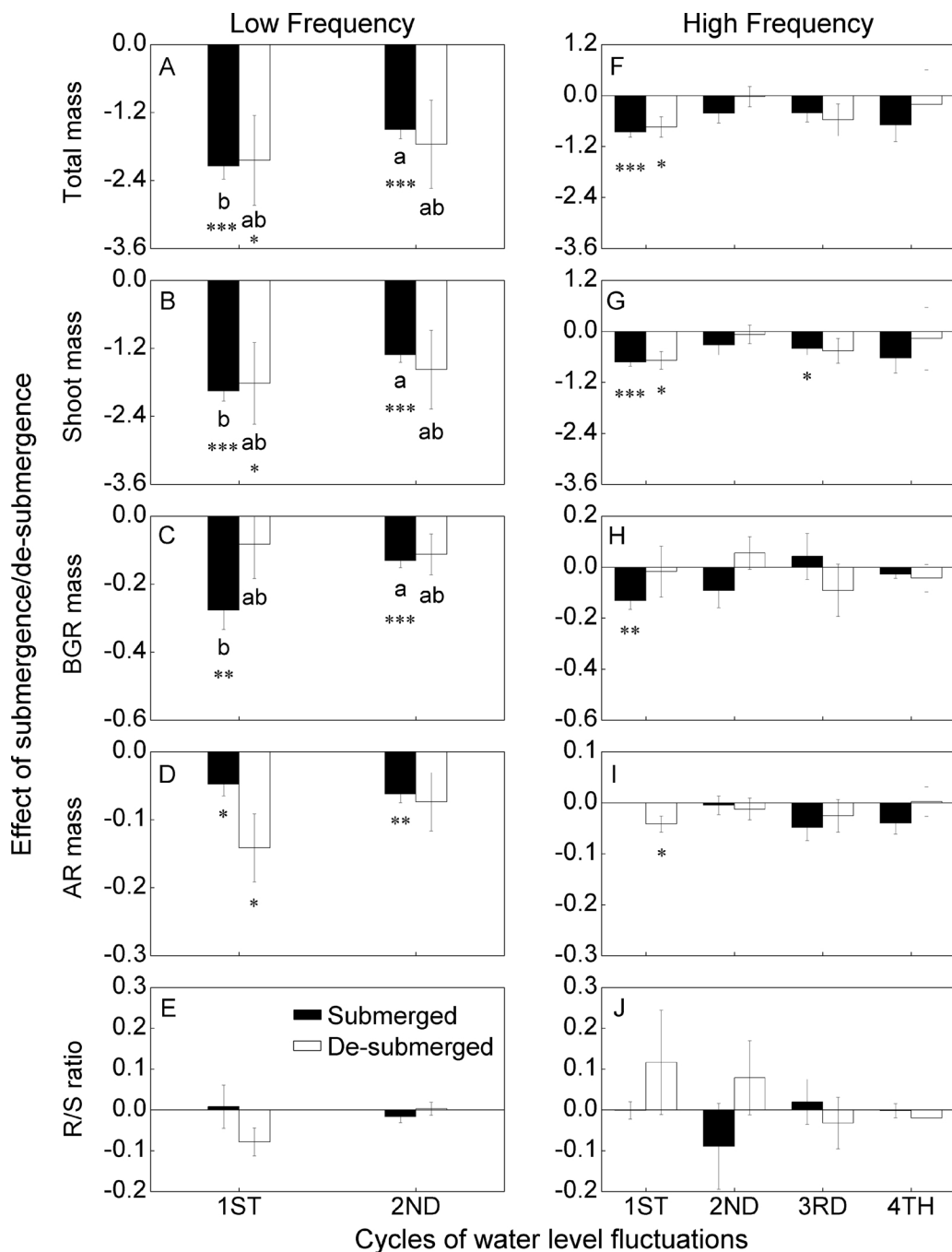


Fig. 3. The relative effects of submergence and de-submergence on growth of *Alternanthera philoxeroides* in low- (A–E) and high-frequency (F–J) water level fluctuations. The relative effects were calculated for total mass (A and F), shoot mass (B and G), belowground root mass (BGR mass; C and H), adventitious root mass (AR mass; D and I) and root to shoot ratio (R/S ratio; E and J) by comparing the treatment vs control and subtracting the difference which existed at the end of the preceding submergence or de-submergence event. Data are mean values (+ s.e., $n = 8$). For the differences between the submergence and de-submergence treatments, means sharing the same letter are not significantly different at $p = 0.05$. Letters are not shown when there was no statistically significant difference between the treatments. Asterisks show the effects that are significantly different from zero (no effect): *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

were significant in the two fluctuation cycles of LFF, albeit less pronounced in the 2nd cycle for shoot, BGR and total mass (Fig. 3A–D). Note that the control plants of the 1st cycle did not experience any submergence, whereas the control plants of the 2nd cycle were recovering from the 1st submergence/de-submergence cycle. Compared to LFF, the relative effects of each five-day submergence event in HFF were smaller and did not significantly change from the 1st to the 4th cycle (Fig. 3F–I). Most of the significant negative effects, i.e., in comparison to zero (no effect), were found after the 1st submergence. The relative effects of de-submergence (open bars) on plant growth were largely comparable with the relative effects of the preceding submergence (Fig. 3). For de-submergence, only the 1st cycle had significantly negative effects in both LFF and HFF (Fig. 3).

3.3. Photosynthetic capacities of plants in LFF and HFF

In parallel to the measurements of biomass accumulation shown above, changes in photosystem II activity were analyzed in the youngest fully expanded leaves. Looking at the maximal photosystem II efficiency, F_v/F_m was significantly lower in the de-submergence treatment than in the control after the 1st cycle but not the 2nd cycle in LFF, while the values declined with every submergence event except in the 3rd cycle in HFF (Fig. 4A and D). Negative effects of submergence were also evident for the effective quantum yield of photosystem II ($\Delta F/F_m'$) in both LFF and HFF (Fig. 4B and E). Unlike F_v/F_m , however, $\Delta F/F_m'$ exhibited more or less full recovery during each de-submergence. The fluorescence indicator of photoprotection, NPQ, remained similar in the treatments and the control during the 1st cycle in both LFF and HFF (Fig. 4C and F). However, it decreased in the treatment plants

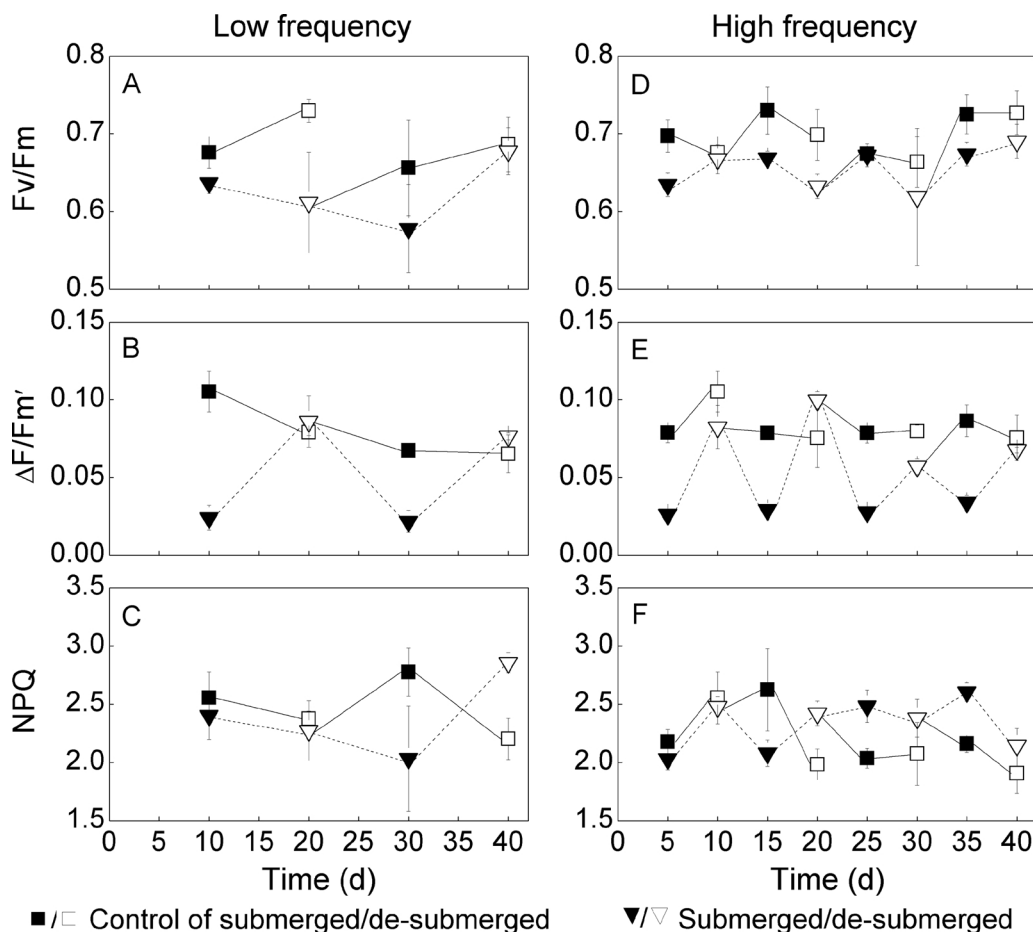


Fig. 4. Changes in maximum (F_v/F_m ; A and D) and effective quantum yield ($\Delta F/F_m'$; B and E) of photosystem II and non-photochemical energy quenching (NPQ; C and F) in leaves of *Alternanthera philoxeroides* during low- (A–C) and high-frequency (D–F) water level fluctuations. The scheme for connecting the symbols is as described in the legend to Fig. 2. Symbols are mean values (\pm s.e., $n = 4$).

during the 2nd submergence before exceeding the levels in the control plants after the 2nd de-submergence in both LFF and HFF. Thereafter, the NPQ level in HFF remained higher in the treatment plants.

3.4. Relative effects of each submergence/de-submergence cycle on photosynthesis

Submergence mostly had negative effects on F_v/F_m and $\Delta F/F_m'$, and also on NPQ during the first two cycles in both LFF and HFF (Fig. 5). In LFF, the negative effects of ten-day submergence on F_v/F_m increased from the 1st to the 2nd cycle, whereas $\Delta F/F_m'$ changed in the opposite direction (Fig. 5A and B). For these two parameters of photosystem II efficiency, the negative effects of five-day submergence did not significantly change in HFF during the entire experiment (Fig. 5D and E). In contrast to submergence, de-submergence often had positive effects on F_v/F_m , $\Delta F/F_m'$ and NPQ, reflecting the recovery from the negative effects of the preceding submergence (Fig. 5). In the case of $\Delta F/F_m'$, the recovery effects during de-submergence were observed already in the 1st cycle (Fig. 5B and E). As for NPQ the effects of de-submergence became mostly positive in both LFF and HFF after the 2nd cycle (Fig. 5C and F).

3.5. Effects of fluctuation frequency on plant growth and photosynthesis

With the same total duration of submergence and de-submergence, the plants in LFF and HFF did not show any significant differences in growth and photosynthetic parameters on day 20 (Fig. 6). However, the increase in total mass measured at the end of the experiment (day 40) was twice as high in LFF (+76%) as in HFF (+38%). Specifically, root mass (BGR + AR), $\Delta F/F_m'$ and NPQ were significantly higher in LFF than in HFF on day 40 (Fig. 6C, E and F), suggesting more detrimental

effects of HFF. Still, shoot mass, which accounted for 80–90% of the total mass, did not differ significantly between the plants in LFF and HFF on day 40 (Fig. 6A and B). Also, the plants had similar F_v/F_m values in both conditions (Fig. 6D).

4. Discussion

4.1. Acclimation to consecutive submergence events

Previous studies have found that a single complete submergence event can severely inhibit growth of riparian plants, which is attributed to low light availability and slow gas diffusion in underwater conditions (Colmer et al., 2013; Voeselek et al., 2016; Striker and Colmer, 2017). In this study, we found negative effects of consecutive submergence events on the growth and photosynthetic capacities of *A. philoxeroides* in both LFF and HFF, confirming the first hypothesis about the negative effects for consecutive submergence.

The negative effects of submergence on growth were significantly smaller in the 2nd LFF cycle compared to the 1st LFF cycle, whereas the effects of the four submergence events did not differ significantly in HFF (Fig. 3). The smaller relative impact of the 2nd submergence in LFF can be explained by the limited growth of the control plants which were recovering from the 1st cycle, in combination with the reduced loss of biomass in the submerged plants during the 2nd cycle (Fig. 2). It seems that the plants were less prone to tissue loss in the 2nd submergence event than in the 1st, at least in LFF. This is in accordance with the second hypothesis that assumes less negative impacts of treatments (here submergence) as a result of acclimation.

In HFF, on the other hand, dry weight (except for AR) decreased in the submerged plants not only in the 1st but also in the 2nd and the 4th cycles (Fig. 2). Note that tissue loss (or no growth) was also seen in the

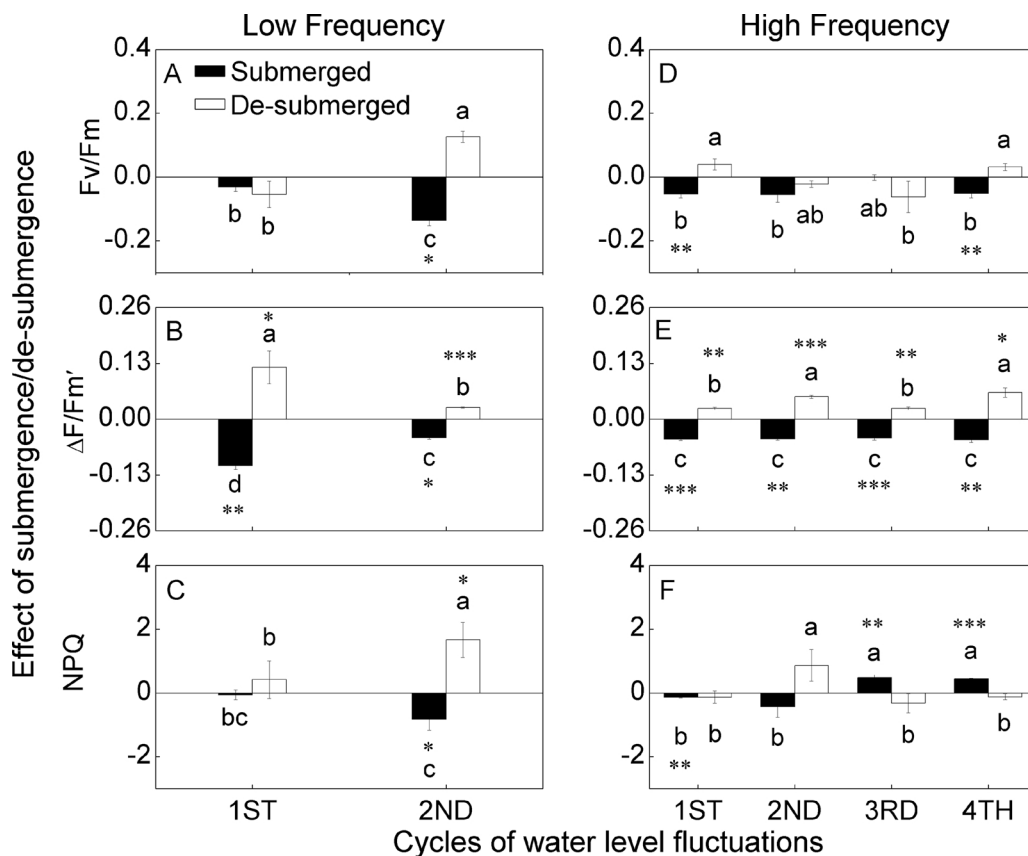


Fig. 5. The relative effects of submergence and de-submergence on photosynthetic capacities of *Alternanthera philoxeroides* in low- (A–C) and high-frequency (D–F) water level fluctuations. The effects were calculated for the maximum quantum yield (Fv/Fm; A and D), the effective quantum yield (ΔF/Fm'; B and E) and non-photochemical energy quenching (NPQ; C and F) in the same way as explained in the legend to Fig. 3. Symbols are mean values (± s.e., n = 4). For the differences between the submergence and de-submergence treatments, means sharing the same letter are not significantly different at p = 0.05. Asterisks show the effects that are significantly different from zero (no effect): *** p < 0.001, ** p < 0.01, * p < 0.05.

control plants of HFF during the 2nd and the 4th submergence events (i.e., during the recovery from the 1st and the 3rd cycle) (Fig. 2). It has been reported that some wetland species show defoliation upon submergence, presumably to reduce respiration (Chen et al., 2013; Striker et al., 2017). After de-submergence, strong decrease in root hydraulic conductivity may cause wilting of shoots and delay photosynthesis recovery (Holbrook and Zwieniecki, 2003; Luo et al., 2016; Ye et al., 2018). Our results are in line with these previous findings but suggest greater difficulty of the plants to maintain tissue and recover growth in HFF than in LFF. For consecutive submergence, the observations in the present study do not support the third hypothesis which predicts better acclimation of *A. philoxeroides* in HFF.

Importantly, the severer growth impairment in HFF (Fig. 3) was apparently not caused by greater limitations or damage to photosynthesis (Fig. 4). As far as ΔF/Fm' is concerned, fluctuation frequency made little difference for *A. philoxeroides* during the treatments. The increase in dissolved O₂ in floodwater at the end of the treatments also indicates high capacity of underwater photosynthesis of the treatment plants. The results may imply that, at the level of photosynthetic electron transport, this species is able to cope with water level fluctuations (Luo et al., 2009; Zhang et al., 2015) regardless of whether at low or high fluctuation frequency. As was seen for growth, the less negative effects found for ΔF/Fm' during the 2nd submergence event of LFF than in the 1st (Fig. 5B) was due mainly to the reduced ΔF/Fm' values

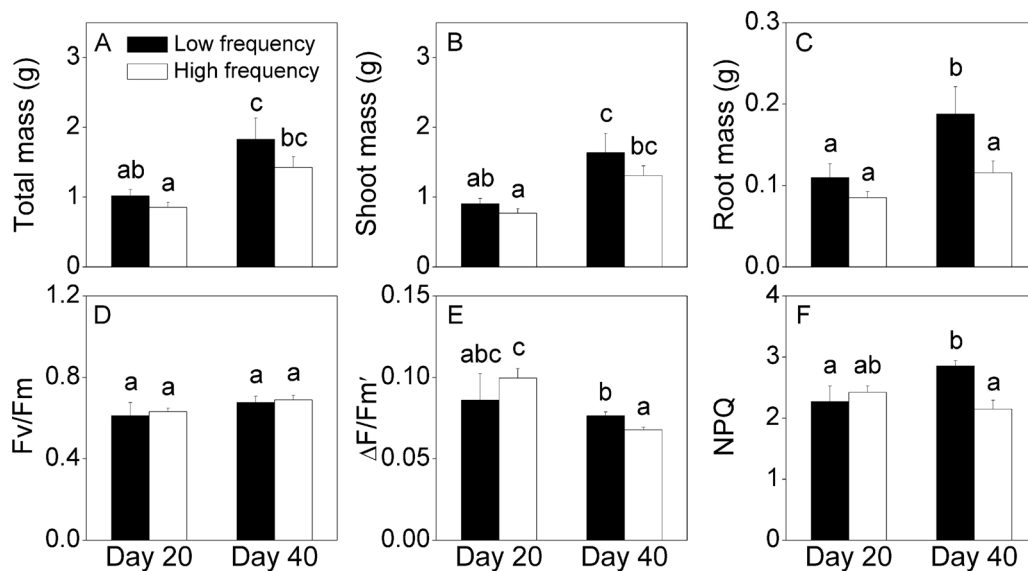


Fig. 6. Changes in growth (A–C) and photosynthesis parameters (D–F) measured in the treatment plants of *Alternanthera philoxeroides* after 20 and 40 days of low- and high-frequency water level fluctuations. Root mass is the sum of belowground roots and adventitious roots. Data are mean values (± s.e.; n = 8 for growth, n = 4 for fluorescence parameters). Means sharing the same letter are not significantly different at p = 0.05.

measured in the control plants rather than improved performance in the submerged plants (Fig. 4B). In fact, $\Delta F/F_m'$ declined to similarly low values in all submergence events in both LFF and HFF (Fig. 4B and E). Nevertheless, maintenance of the electron transport capacity ($\Delta F/F_m'$) during consecutive submergence events, instead of becoming increasingly damaged and impaired with each additional cycle, would require acclimation (Luo et al., 2009; Wang et al., 2016; Yamori et al., 2016).

A clearer sign of acclimation was found for NPQ in HFF, which was enhanced during the 3rd and the 4th submergence event (Figs. 4F and 5F). It seems that HFF induces acclimation of photoprotection in *A. philoxeroides* probably because it is more detrimental than LFF for growth and tissue maintenance. Photoprotective acclimation is typically seen in plants when light energy utilization for CO₂ assimilation and growth cannot keep pace with light energy absorption (Alter et al., 2012). Note that the average daily solar radiation as well as other environmental conditions were mostly comparable between the first two and the last two submergence periods in HFF (Supplementary Table S1). Hence, the up-regulation of NPQ found in the submerged plants during the later HFF cycles most likely reflects progressive photoprotective acclimation triggered by repeated submergence.

Taken together, consecutive submergence does have negative effects on growth and photosynthesis of *A. philoxeroides* but it also induces acclimation, as exemplified by reduced tissue loss during the 2nd submergence in less stressful LFF (Fig. 2A–D) or increased NPQ levels measured after the 3rd and the 4th submergence in more stressful HFF (Fig. 4F). Also the maintenance of $\Delta F/F_m'$ during the consecutive submergence events in LFF and HFF (Fig. 4B and E) would be difficult without acclimation.

4.2. Acclimation to consecutive de-submergence events

Post-submergence effects following a single submergence event can limit plant growth because de-submerged plants suffer from injuries that developed during and after submergence (Panda et al., 2008; Tamang et al., 2014; Liu et al., 2015). Separating the effect of each de-submergence event from that of the preceding submergence event, the results showed that consecutive de-submergence had negative effects on the growth of *A. philoxeroides* in both LFF and HFF (Fig. 3A–D), which provides another support to the first hypothesis. Furthermore, we found that the relative growth impacts of de-submergence were largely comparable with those of submergence (Fig. 3), highlighting the significance of de-submergence as a hurdle for plant survival and performance during water level fluctuations (Striker, 2012; Striker et al., 2017).

The shoot mass and the total mass increased in both control and de-submerged plants during the 1st and the 2nd de-submergence events of LFF, whereas such increase in biomass was not always seen in HFF (Fig. 2). This corresponds to the greater tissue loss of submerged plants found in HFF. Increased injuries and tissue loss during submergence could have impeded growth recovery after de-submergence, resulting in the lower shoot and root mass of the treatment plants in HFF than in LFF (Fig. 6). Similarly, wetland species *Chloris gayana* was shown to accumulate 2.9-fold higher dry mass when it was subjected to a single 2-week submergence compared to 1-week submergence twice (Striker et al., 2017). High environmental fluctuations, as was the case in HFF in the present study, seem to pose a great challenge to plants (Garssen et al., 2015; Baastrup-Spohr et al., 2016; Striker et al., 2017), probably because they necessitate rapid and frequent acclimation and re-acclimation.

Neither the biomass nor the fluorescence data showed less severe effects of de-submergence during later cycles (Figs. 3–6), which once again denies the second hypothesis about diminishing negative effects of de-submergence through acclimation. However, the plants were able to modulate the photosynthetic parameters dynamically in both LFF and HFF (Fig. 4), which may have been essential to cope with changing conditions (Luo et al., 2009; Wang et al., 2016; Yamori et al., 2016).

Despite the equally dynamic adjustments of the photosynthetic parameters in LFF and HFF, post-submergence recovery of growth was more severely restricted in HFF, suggesting increased costs for healing injuries and decreased net carbon gain in smaller shoots. It is also possible that de-submerged (as well as submerged) plants in HFF contained a smaller number of functional photosystems per unit leaf mass, as has been shown for photosynthetic pigments; the leaf pigment content of *C. gayana* decreased more strongly in HFF than in LFF (Striker et al., 2017).

On the whole, de-submerged plants of *A. philoxeroides* were not doing better in HFF than in LFF, thus casting a doubt on the third hypothesis. Yet, reduced growth performance in both LFF and HFF by no means excludes effective induction of acclimation. If it were not for acclimation, the plants may not have survived the submergence/de-submergence cycles in our experiment. As discussed above for submerged plants, acclimation may be manifested by different parameters in LFF and HFF, depending on the severity of stress and injuries.

5. Conclusions

Consecutive submergence and de-submergence have comparable negative effects on growth of *A. philoxeroides* while they induce contrasting responses (down- and up-regulation, respectively) of photosynthetic electron transport, resulting in dynamic variations of the photosynthetic parameters during water level fluctuations. Whether the impacts of submergence and de-submergence diminish with additional fluctuation cycles or not, the answer seems to depend on the severity of stress and injuries on the one hand and the acclimation capacity of plants on the other hand. Although HFF enhances tissue damage and thus restricts growth of *A. philoxeroides* more strongly than LFF, both HFF and LFF effectively induce acclimation in this plant which survives these treatments successfully. Physiological mechanisms and metabolic alterations involved in acclimation to consecutive submergence and de-submergence deserve further investigations.

Declaration of contributions

Conception and design of the study: Fang-Li Luo and Fei-Hai Yu.
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 Critical revision of the article for important intellectual content and final approval of the article: Shizue Matsubara and Ming-Xiang Zhang.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at <https://doi.org/10.1016/j.envexpbot.2018.08.015>.

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