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Leaf gas exchange, chlorophyll fluorescence and growth responses of *Melaleuca alternifolia* seedlings to flooding and subsequent recovery

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Abstract

Periodic flooding of trees in tropical floodplains and reservoirs where water levels fluctuate is a common phenomenon. The effects of flooding and subsequent recovery on gas exchange, chlorophyll fluorescence and growth responses of *Melaleuca alternifolia* seedlings, a tall shrub species used in floodplain and reservoir forest restoration in southern China, were studied during a grow season (from March to December in 2007). *M. alternifolia* seedlings were flooded for 180 days, drained and left to recover for another 60 days. Survival rates of the seedlings were 100% during the 180-day flooding period. Chlorophyll (Chl) content, net photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration rate (E) of the flooded seedlings were all significantly lower than those of the control. Significant reductions of photochemical quenching coefficient (q_p) and increases of nonphotochemical quenching (NPQ) in the flooded seedlings were observed. However, there were no significant differences in the maximal quantum yield of PSII photochemistry (F_v/F_m) between treatments. All seedlings survived during the two-month recovery period after the flooded treatment was drained, and the biomass and height of the recovered seedlings approached those of the control at the end of the experiment. During the first-month recovery period, Chl content, P_N, g_s and E in the recovered seedlings were all obviously low, then increased gradually and rose to the levels similar to the control by the end of the experiment. Quenching analysis revealed significant reductions of q_p and increments of NPQ in the recovered seedlings at the beginning of draining, and a nearly complete recovery for both parameters by the end of the experiment. However, F_v/F_m of the recovered seedlings did not differ significantly from the control during the recovery period. Our study demonstrated that *M. alternifolia* seedlings can survive and grow through 180 days of flooding with a subsequent 60-day recovery period in drained conditions, indicating that seedlings of this species would be suitable for afforestation in areas exposed to intermittent flooding.

Additional key words: chlorophyll fluorescence; flooding; leaf gas exchange; *Melaleuca alternifolia*; recovery.

Introduction

Flooding changes a number of physical, chemical and biological properties of the soil, altering drastically the environmental conditions for tree growth (Pezeshki and DeLaune 1998, Parolin *et al.* 2006, Kramer *et al.* 2008). For many tree species, flooding may adversely affect plant physiological functioning such as nutrition, water relations, the activity of photosynthetic enzymes, gas exchange, photosynthetic electron transport, and photosystem II activity. However, flood-tolerant tree species possess physiological, metabolic, morphological, and anatomical adaptations to survive the detrimental effects of the anaerobic soil environment created by flooding (Pezeshki *et al.* 1996, Kozlowski 2002, Stewart *et al.* 2007, Iwanaga and Yamamoto 2008).

Flooding and subsequent recovery of the vegetation in tropical floodplains and reservoirs with fluctuating water levels is a common phenomenon. The alternating terrestrial and aquatic periods with advancing and receding

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Abbreviations: Chl – chlorophyll; E – transpiration rate; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; q_p – photochemical quenching coefficient; PPFD – photosynthetic photon flux density.
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floods poses a variety of constraints to the plants growing in this habitat, including oxygen starvation, accumulation of reduced compounds in the soil, sedimentational and erosional processes. If flooding is recurrent before the plants recover from previous flooding, injuries will accumulate and serious damage may occur. Therefore, when assessing plant resistance to flooding, it is important to consider the ability of plants to recover from transient waterlogging. The success in tree planting on floodplain and reservoir depends not only on the trees' tolerance to submersion, but also on their subsequent recovery (Loucks and Keen 1973). Previous studies on physiological and growth responses of tree species to flooding have mostly focused on the responses to continuous flooding (Tsukahara and Kozlowski 1985, Parolin 2001, Naumann *et al.* 2008). Little is known about the responses to subsequent recovery after the removal of the flooding stress.

Melaleuca alternifolia, a member of the Myrtaceae family, is a fast-growing tree native to Australia. *M. alternifolia* develops into a tall shrub with paperbark and produces tea tree oil, a commodity in demand by the health-care industry. *M. alternifolia* grows successfully under waterlogged conditions and is used in constructed wetlands for wastewater treatment (Bolton and Greenway 1997, Bolton and Greenway 1999a, Bolton and Greenway 1999b). *M. alternifolia* was introduced to China in 1980s and there have not been any detrimental impacts resulting from the introduction of this non-native species. Attempts to use *M. alternifolia* in floodplain and reservoir afforestation have recently been made in Guangdong Province and Guangxi Province, China, where the annual flood period lasts three to six months with extremely high water levels. At the moment there have been no studies addressing the effects of soil flooding and subsequent recovery on the growth and eco-physiological characteristics of *M. alternifolia* seedlings.

The objectives of this study were (*1*) to characterize the impacts of flooding on gas exchange, chlorophyll fluorescence and growth responses, and (*2*) to determine the extent of recovery of several key physiological parameters after the removal of flooding stress.

Materials and methods

Plant materials and experimental design: An outdoor completely randomized experiment was conducted in South China Normal University, Guangzhou (23°08' N and 113°09' E), China from March to December in 2007. During the experiment, air temperature and relative humidity ranged from 19 to 37°C and 31% to 96%, respectively. On March 2, 2007, one-year-old *M. alternifolia* seedlings (115±7.5 cm in height, 25±1.3 mm in diameter at a base) grown at the field of our campus were transplanted to buckets (60 cm depth, 40 cm diameter, one plant per bucket) containing 15 kg well-mixed forest soil. All seedlings were exposed to direct sunlight and natural rainfall, and fertilized biweekly with 1/2 strength Hoagland's solution. On April 3, 2007 after transplanting to the buckets for a 30-d period of acclimation, a total of 200 seedlings of uniform size were selected and assigned randomly into two groups of 100 seedlings. Seedlings were subjected to two hydrological treatments: (*1*) control, which was watered and well drained; and (*2*) flooded and subsequently drained. In the control treatment, excess water was allowed to drain from the bucket through a hole 1 cm above the bottom of the bucket. In the flooded and subsequently drained treatment, water level was maintained at 20 cm above the soil surface for 180 days. After the flooding period was complete, water was drained and this treatment was thereafter handled the same as the control treatment for an additional 60 d. The ambient conditions varied at the sampling time throughout the experiment, but the conditions of measurement were in all cases the same between the control and the treatment.

Photosynthesis and Chl fluorescence: Measurements were taken on mature leaves (from an intermediate position on the stem) in each of the 10 replicate plants once every 15 days. Chl content was extracted from about 1 g of leaf on 80% acetone. Total Chl and Chl *a* and *b* were estimated by a procedure described by Lichtenthaler and Wellburn (1983). P_N, E and g_s were measured using a portable infrared gas analyzer (*LICOR-6400, LI-COR Inc.*, Lincoln, USA) during 9:00–11:30 h. In the measurements, CO_2 concentration was controlled at 370 μmol mol^{-1} with *LI-COR* CO_2 injection system, and a saturating photosynthetic photon flux density (PPFD) of 800 μmol m^{-2} s^{-1} from a *LI-COR* LED irradiation source was supplied. Air temperature of leaf chamber was maintained at about 30ºC. Before recording of data, the measured leaves were kept in the leaf chamber for at least 5 min to reach a steady state of photosynthesis. During the leaf gas exchange measurements, Chl fluorescence was measured simultaneously with a pulse-modulated fluorometer (*PAM 2100, Walz*, Effeltrich, Germany). Prior to each measurement, a clip was placed on the leaf for 30 min for dark adaptation. The weak-modulated irradiance, “actinic light”, and saturating pulse were 0.05, 200, and 5,000 μmol m^{-2} s^{-1}, respectively. F_v/F_m, q_p and NPQ were calculated according to Maxwell and Johnson (2000).

Plant growth: Tree heights were measured monthly with a ruler, and 10 complete trees from each treatment were harvested once every month, and weighed after drying for 48 h in a forced air oven at 80°C.

Statistical analysis: All statistical analyses were performed with *SPSS 11.0* and *Excel 2003 for Windows*. Repeated measurement analysis of variance (*ANOVA*) was used and differences were considered significant at $p<0.05$.

Results

Growth and physiological responses during the flooded period: Throughout a 180-d flooding period, the survival rates of seedlings were 100%, with no visible damage symptoms in the flooded seedlings. Formation of adventitious roots and hypertrophied lenticels were observed in the flooded seedlings. Stem lenticels on the submerged portion showed hypertrophic development in a few days after the initiation of flooding (data not shown). Only a few trees (about 12%) formed adventitious roots at submerged portions of stems after 30 d of flooding, and all trees developed adventitious roots after flooding treatment for 90 days. No significant ($p>0.05$) reductions in the biomass and height of the flooded seedlings were observed within 60 d of flooding, but significant ($p<0.05$) reductions in both these parameters were observed after 60 d of flooding, with 12% and 14% reduction in biomass and height, respectively, at the end of the flooding experiment (Fig. 1*A,B*).

Chl content, P_N, g_s and E all decreased significantly ($p<0.05$) during the flooding period, with 13%, 19%, 21% and 16% lower than the control, respectively (Figs. 2*A,B,C*; 3*A,B,C*), suggesting that photosynthetic performance of the plants was influenced by the flooding. Throughout the flooding period, the F_v/F_m values were often greater than 0.80 in all the seedlings. These measurements were relatively stable and very similar in the flooded as well as in the control plants (Fig. 4*A*), indicating an undamaged photochemical apparatus based on the F_v/F_m values which are close to 0.83 for 'healthy' plants (Maxwell and Johnson 2000). There were no significant reductions in q_p and increases in NPQ within 30 days of flooding (both $p>0.05$). However, significant reductions (both $p< 0.05$) in q_p and increments in NPQ after 30 d of flooding were also observed. The mean q_p and NPQ values for the flooded plants were 95% and 103% of control (Fig. 4*B,C*).

Growth and physiological responses during the recovery period after draining: When the water receded, adventitious roots were exposed to air, dried and

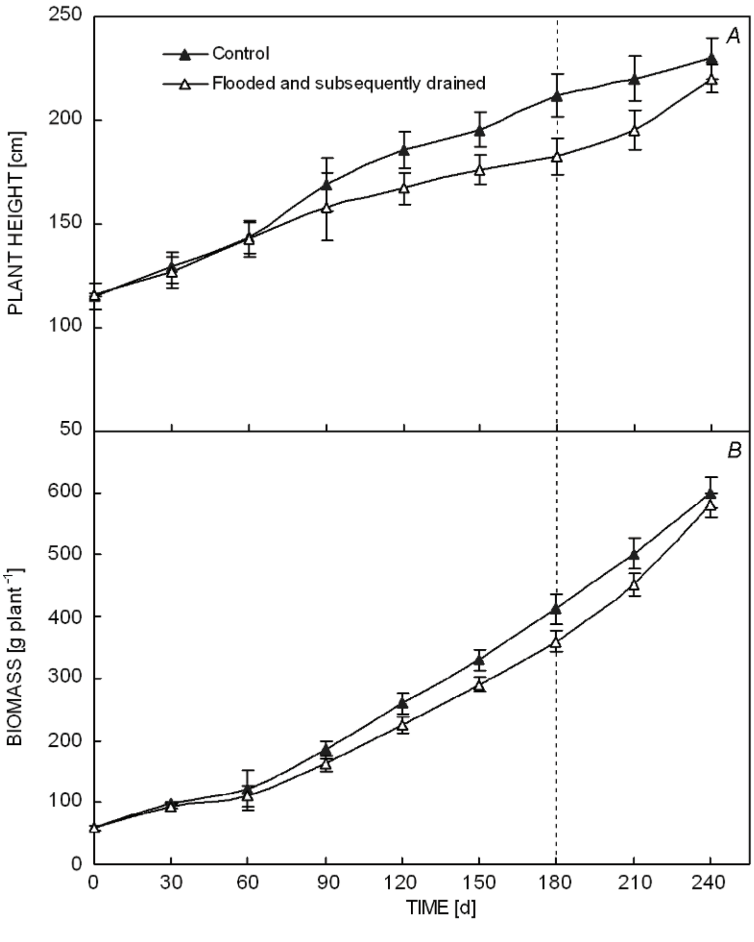

Fig. 1. Changes of height (*A*) and biomass (*B*) in *M. alternifolia* seedlings during the flooded period (day 0 to day 180) and the recovery period after draining (day 180 to day 240) (Means±SD, $n = 10$).

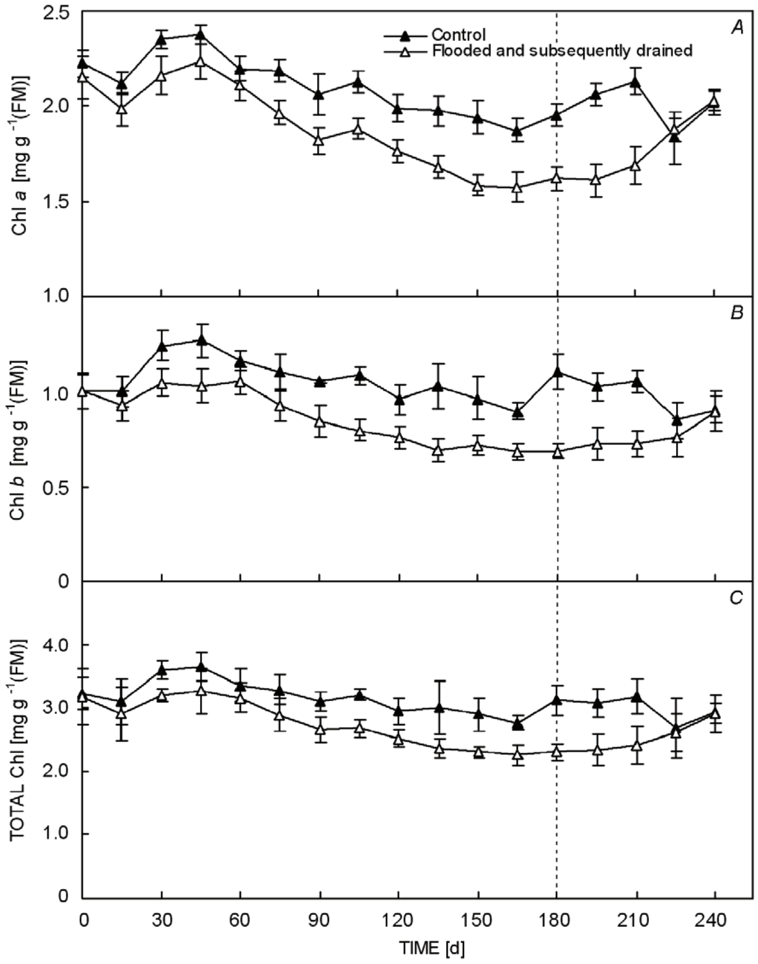

Fig. 2. Changes of chlorophyll (Chl) *a* (*A*), Chl *b* (*B*) and total Chl (*C*) content in *M. alternifolia* seedlings during the flooded period (day 0 to day 180) and the recovery period after draining (day 180 to day 240) (Means±SD, *n* = 10).

died. At the same time, seedlings regenerated new underground roots. All seedlings survived during two-month recovery period after the flooded treatment was drained, the height and biomass of the recovered seedlings increased gradually and were about 86% and 87% of control at the beginning of draining, 89% and 90% at 30 days after draining, and 95% and 96% at the end of the experiment, respectively (Fig. 1*A,B*).

During the first month after draining, Chl content, P_N, g_s and *E* of the recovered seedlings were all significantly lower than those of the control treatment ($p<0.05$), then increased gradually and recovered to the levels similar to the control by the end of experiment (Figs. 2*A,B,C*; 3*A,B,C*), which indicates rapid recovery of photosynthetic activity in response to the removal of the flood-water. Similarly to the results found during the flooded period, F_v/F_m changed slightly and was not significantly ($p>0.05$) different from the control (Fig. 4*A*). At the beginning of draining, q_p was significantly lower ($p<0.05$), and rose to the level of the control by the end of the experiment (Fig. 4*B*). In contrast, NPQ was high at the beginning and gradually decreased to the level of the control (Fig. 4*C*).

Discussion

The adventitious roots support shoot growth during prolonged waterlogging period by supplying water, minerals and hormones (Senagomes and Kozlowski 1980, Tsukahara and Kozlowski 1985, Islam and Macdonald 2004). Lenticels are the important pathway of gas exchange between atmosphere and internal tissues in stems and roots (Kozlowski and Pallardy 2002). Hypertrophied lenticels with abundant intercellular spaces in flooded plants can enhance gas diffusion and provide oxygen to the submerged roots (Yamamoto *et al.* 1995). Moreover, owing to the anaerobic metabolism of plants, significant accumulation of toxic substances occurs in waterlogged soil (Armstrong and Armstrong 2001, Pang *et al.* 2007). The adventitious roots oxidize the rhizosphere, facilitating the transformation of some soil-borne toxins into less harmful compounds, and increase the supply of root-synthesized gibberellins and cytokinins to the leaves (Kozlowski 1997). Although the height and biomass were affected by flooding (Fig. 1), our results indicate that *M. alternifolia* exhibited considerable

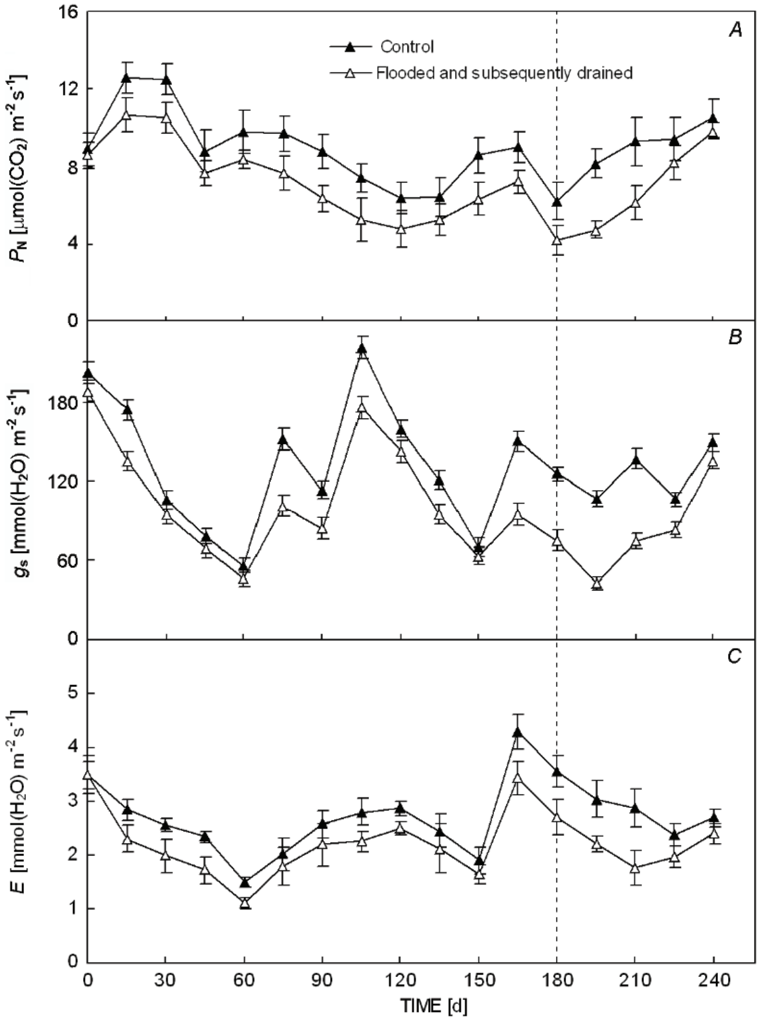

Fig. 3. Changes of net photosynthetic rate, P_N (*A*), stomatal conductance, g_s (*B*) and transpiration rate, *E* (*C*) in *M. alternifolia* seedlings during the flooded period (day 0 to day 180) and the recovery period after draining (day 180 to day 240) (Means±SD, *n* = 10).

morphological adaptations to flooding by producing hypertrophied lenticels and abundant adventitious roots on submerged portions of stems, which are likely the key processes that enable *M. alternifolia* to withstand flooding.

Decreases in photosynthesis and stomatal conductance under the flooding condition have been demonstrated in many woody species (Gravatt and Kirby 1998, Senagomes and Kozlowski 1988). In the present experiment, gas exchange decreased during flooding, but recovered rapidly following drainage (Fig. 3), which indicates that decreased gas exchange was temporary when exposed to soil flooding. Rapid recovery of photosynthesis after the flooding is important for the survival of the flooded woody plants in wetland forests (Pezeshki 1994). In the present study, gas exchange and growth recovered to levels similar to the control after 60 days of draining (Figs. 1, 3), which indicated that *M. alternifolia* possessed rapid recovery ability after the flood-water was drained. The ability of this species to recover swiftly from reduced photosynthetic activity may enable it to survive and to grow in periodically flooded areas such as tropical floodplain and reservoir.

Chl fluorescence is a very sensitive indicator for the stress-induced damage to photosystem II (Maxwell and Johnson 2000). F_v/F_m is thought to indicate the effects of environmental stresses on photosynthesis (Ball *et al.* 1994, Jones *et al* 2006, Lavinsky *et al.* 2007). A decline in F_v/F_m is a good indicator for photoinhibitory impairment resulting from waterlogging stress. The maintenance of a stable F_v/F_m indicates no damages to photochemical reactions (Mielke *et al.* 2003). However, F_v/F_m is not always a sensitive indicator for the effects of environmental stresses on photosynthesis. Although significant reductions in the rate of CO$_2$ assimilation were observed, F_v/F_m remains not significantly affected in the stressed leaves (James *et al.* 2002, Mielke *et al.* 2003). Throughout the experiment, F_v/F_m values were close to 0.82 and very similar in the flooded as well as in the control plants (Fig. 4*A*), indicating that the photochemical reactions were not damaged and F_v/F_m was not a sensitive indicator for the effects of flooding on photosynthesis in*M. alternifolia*. NPQ measures the efficiency of thermal energy dissipation (Maxwell and Johnson 2000) and thus the amount of energy that is not used in photochemistry. In the present experiment, we also analyzed NPQ that

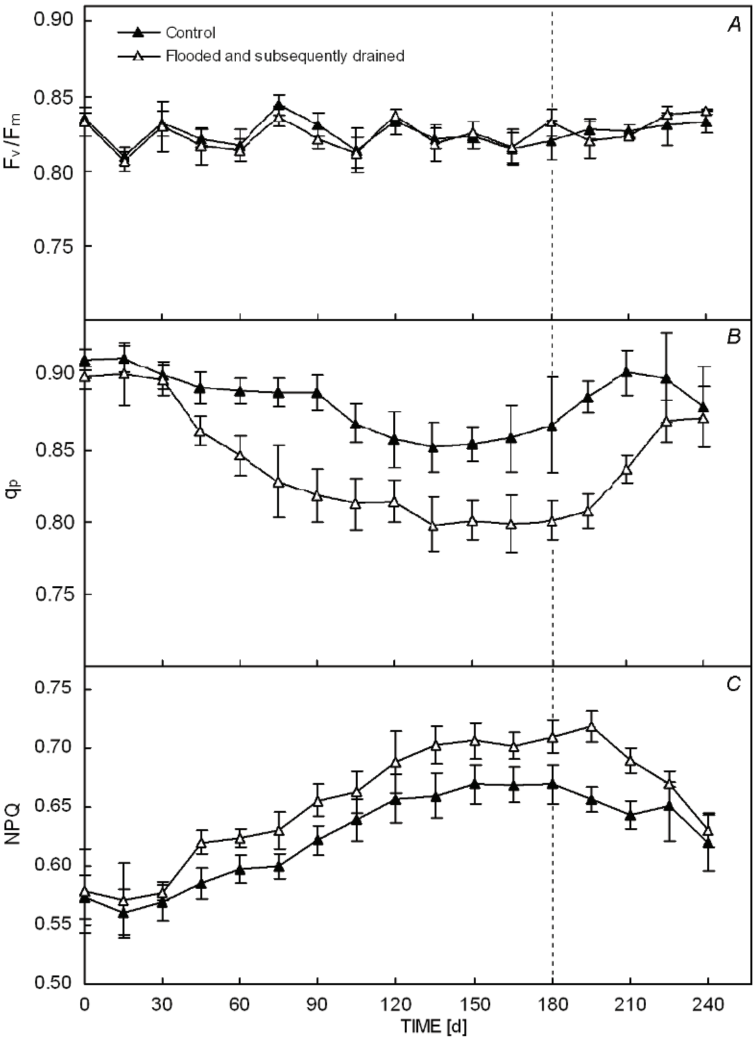

Fig. 4. Changes of the maximum quantum efficiency of the photosystem II, F_v/F_m (*A*), photochemical quenching coefficient, q_p (*B*) and non-photochemical quenching, NPQ (*C*) in *M. alternifolia* seedlings during the flooded period (day 0 to day 180) and the recovery period after draining (day 180 to day 240) (Means±SD, *n* = 10).

increased significantly during flooding and the beginning of draining, and a nearly complete recovery by the end of experiment (Fig. 4*C*). These results suggest that NPQ may be even more sensitive than F_v/F_m to the flooding effect on leaf photosynthetic performance in *M. alternifolia*, at least under our experimental conditions.

The longer trees are exposed to flooding, the greater the potential for injury. Most trees can withstand only 1–4 months with water being continuously over the soil surface. Compared to the tolerance threshold of a 40–45% flooded vegetation period observed by Hall and Smith (1955), *M. alternifolia* that survived a 180-d flooding period during this experiment exhibited excellent flooding tolerance. At present, laboratory experiments that are mostly carried out on seedlings, provide important insights into the mechanisms of flooding tolerance. However, it is difficult to apply these results to adult trees or the *in situ* conditions. In order to achieve a better understanding of the flooding tolerance of this tree species, findings from the laboratory experiments must be compared to and validated with field experiments.

In summary, outdoor cultivated seedlings of *M. alternifolia* can survive and grow through a period of 6 months of soil flooding, despite reductions in gas exchange and growth rates. No damage to the photochemical apparatus of photosynthesis was observed under the flooded conditions. Gas exchange and growth recovered to the levels similar to the control after 60 days of draining. Based on the results we conclude that seedlings of this species would be suitable for use in afforestation projects in areas exposed to intermittent flooding.

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