Post-submergence recovery of photosynthesis and growth: A comparison between two wetland plants

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To my parents

Summary

Flooding, including complete submergence as the most extreme case, is one of the common environmental challenges for plants in natural and artificial ecosystems. Recent studies, especially those in rice, have advanced our understanding of the regulatory mechanisms induced in plants during flooding. In comparison, recovery processes and responses upon de-submergence are not so well understood, even though plant performance during flood intervals is critical for their establishment in water-level-fluctuation zones.

In this PhD thesis, the behavior of two wetland species, *Alternanthera philoxeroides* and *Hemarthria altissima*, was studied during recovery following complete submergence. In an attempt to infer the strategies of submergence tolerance in these plants, I first checked the morphological and anatomical responses during submergence. Following de-submergence, changes in antioxidative defense, photosynthesis, carbohydrate partitioning and growth were closely analyzed both in shoots and roots. Additionally, low-light stress, instead of submergence stress, was applied to some plants to identify the responses and effects which are specifically induced by submergence (low light + low O_2).

Plants of *A. philoxeroides* were characterized by enhanced stem elongation, a typical feature of the escape strategy, and dramatic decrease in photosystem II activities upon submergence. Rapid stem elongation, together with the high porosity in shoots and roots, may improve light environment and O_2 and CO_2 availability of *A. philoxeroides* during submergence, while it leads to carbohydrate depletion when leaves fail to grow out of water. Following de-submergence, *A. philoxeroides* were able to quickly adjust the capacity of photosynthesis and antioxidative defense, which probably is crucial for growth recovery of this species. Plants of *A. philoxeroides* flexibly allocated the biomass to the organs for acquisition of most limiting resources, both during submergence (stems to gain light, O_2 and CO_2) and after de-submergence (leaves to gain carbohydrate).

In contrast, submerged plants of *H. altissima*, having the quiescence strategy, exhibited neither stem elongation nor strong photosystem II downregulation but stored carbohydrate for a longer period. Unlike *A. philoxeroides*, leaves of *H. altissima* could maintain high antioxidant capacities, which may explain the lack of O_2^- and H_2O_2 detection under all conditions. Notably, carbohydrate (sucrose) stored in shoots was rapidly hydrolyzed and utilized to promote growth shortly after de-submergence. In contrast to *A. philoxeroides*,

conservative biomass allocation to culms, a storage organ of this species, may be essential for *H. altissima* to survive future flooding.

Based on these results, I propose that submergence tolerance of the escape and quiescence strategies entails not only the corresponding regulation of carbohydrate catabolism and energy metabolism during submergence but also coordinated regulation of antioxidative defense, photosynthesis, carbohydrate partitioning and growth following de-submergence. The findings shed a new light on submergence tolerance in plants and emphasize the importance to understand whole-plant responses to changing environments when considering stress tolerance.

Table of Contents

Citation of publications in this dissertation

The publications belonging to this cumulative dissertation are cited in the following way. Submitted manuscripts are labelled as "submitted" followed by the year of submission. The complete references are shown as below.

#	Citation	Journal	Status
1	Luo *et al*., 2009	Annals of Botany	published
2	Luo *et al*., 2010	Annals of Botany	in press
3	Luo *et al*., 2010 submitted	Annals of Botany	submitted

Luo F-L, Nagel KA, Zeng B, Schurr U, Matsubara S. 2009. Photosynthetic acclimation is important for post-submergence recovery of photosynthesis and growth in two riparian species. *Annals of Botany,* **104**: 1435–1444.

Luo F-L, Nagel KA, Scharr H, Zeng B, Schurr U, Matsubara S. 2010. Recovery dynamics of growth, photosynthesis and carbohydrate accumulation after de-submergence: A comparison between two wetland plants showing escape and quiescence strategies. *Annals of Botany*, in press.

Luo F-L, Thiele B, Janzik I, Zeng B, Schurr U, Matsubara S. 2010. How do antioxidative defense systems respond to de-submergence in wetland plants having escape and quiescence strategies? *Annals of Botany*, submitted.

1. Introduction

After completion of the Three Gorges Dam, the world's biggest dam to regulate water flow in Asia's longest river, a huge 350 km^2 water-1eve1-fluctuation zone will appear along the Yangtze river (Wang *et al.*, 2008a, b). Since the planning of the dam, questions have been raised concerning its possible impact on the environment, including landscape quality and conservation of soil, water and biodiversity (Stone, 2008). Besides, global climate change is expected to increase frequency and severity of flooding events, which threatens terrestrial plants distributed in flood plains and river deltas (Arnell and Liu, 2001).

Partial to complete submergence is detrimental for many terrestrial plants, causing ultimate death (Voesenek *et al.*, 2006). Submergence has a severe negative influence on the productivity of arable farmland because most crops are not selected to cope with flooding stress (Liao and Lin, 2001). In deepwater and floating-rice areas, flash floods caused by either heavy rains or outflow of nearby rivers affect more than 220000 km^2 of rain-fed lowlands in South and South-East Asia. Especially, modern high-yielding rice varieties always suffer seriously from flooding injuries (Das *et al*., 2009). However, some plant species are able to endure complete submergence, or even grow vigorously in response to submergence (Mommer *et al*., 2005b; Voesenek and Bailey-Serres, 2009). Such variation in flood tolerance is closely correlated with the abundance and distribution of species in flood-prone areas (van Eck *et al*., 2004); relatively flood-tolerant species occur at low elevations along the floodplain while more flood sensitive species are restricted to high elevations of the floodplain gradient.

1.1 Stress during submergence

Water itself is chemically harmless to plants. Nevertheless, it can inflict serious injuries on plants (Jackson and Ram, 2003). The negative impacts of submergence on terrestrial plants are mainly related to low light intensity and slow gas diffusion, which severely restricts carbohydrate and energy availability for plants underwater (Bailey-Serres and Voesenek, 2008; Colmer and Voesenek, 2009).

Severe shading Poor light transmission through floodwater is an important limiting factor for plants in deep water, in highly turbid floodwater or under thick algal growth (Whitton *et al*., 1988). Under these conditions, light reaching the submerged plants/leaves is attenuated by

water, dissolved organic matter, silt, and/or phytoplankton suspended in the water (Das *et al*., 2009). Due to the attenuation, only a scanty amount of solar radiation reaches the plant canopy level, and thus limiting the capacity for underwater photosynthetic carbon fixation (Setter *et al*., 1995). Light limitation during submergence has been shown to cause severe injuries and accelerate plant mortality (Adkins *et al*., 1990; Jackson and Ram, 2003).

Slow rates of gas exchange Rapid diffusion of O_2 to mitochondria enables respiration and CO_2 influx to chloroplasts is required for photosynthesis. The solubility and diffusion coefficient of O_2 decrease 33 and 8.8×10^3 times, respectively, in fully aerated water at 25°C compared with the situation in air (Armstrong, 1979). Compared with O_2, access to CO_2 underwater is favored by the property of CO_2 gas, being relatively soluble in water; the ratio of dissolved CO_2 relative to gas-phase CO_2 in water is approx. 0.76 at 25°C, depending on the pH values of the water (Sisler and Wood, 1988). Nevertheless, photosynthesis of submerged leaves is severely CO_2-limited because of slow gas diffusion in water and the inevitable boundary layer formed around leaves (Setter *et al*., 1989). Slow entry of CO_2 into leaves typically limits photosynthesis when the shoot is fully submerged, whereas CO_2 levels can increase in submerged roots (Mommer and Visser, 2005). Furthermore, regulation of endogenous concentration of the volatile hormone ethylene depends strongly on the rate of its outward diffusion, which also is severely hampered in water (Armstrong, 1979). As a consequence, levels of ethylene typically increase in submerged plant tissues.

1.1.1 Carbohydrate starvation

Soluble sugars and starch in plants are important sources of energy needed for cellular metabolism and maintenance during submergence as well as subsequent regeneration of new tissues after de-submergence (Das *et al*., 2005; Panda *et al*., 2008). Some flood-tolerant species have the ability to retain carbohydrate pools during submergence, provided that they are capable of underwater photosynthesis and/or carbohydrate consumption can be reduced during submergence (Das *et al*., 2005). Underwater photosynthesis of terrestrial plants is limited not only by low light intensity and poor CO_2 availability as described above, but also by structural and functional impairment of chloroplasts (Panda *et al*., 2006; Voesenek *et al*., 2006). Table 1 summarizes photosynthetic and metabolic responses of terrestrial plants to sub-

TABLE 1. Literature overview of the responses of photosynthesis, growth and carbohydrate catabolism in terrestrial plants upon submergence or O_2 deficiency

Responses upon submergence	Species	References
Photosynthesis (adverse impacts)		
low light and low CO_2; sub-optimal pH and temperature	many terrestrial plants	Panda *et al.* (2006) Sarkar *et al*. (2006)
chlorophyll degradation	rice cultivars some Amazonian floodplain trees	Parolin (1997) Sarkar *et al.* (2001) Panda *et al.* (2006)
reduction of Rubisco activity; inactivation of O_2 evolving complex; reduced electron acceptor pool	rice cultivars	Panda *et al.* (2006) Panda *et al.* (2008)
decrease in maximal photochemical efficiency of photosystem II; highly reduced photosynthetic capacities	rice cultivars *Eleocharis cellulose*	Macek *et al.* (2006) Panda *et al.* (2006) Panda *et al.* (2008)
Photosynthesis (acclimation)		
improvement of O_2, CO_2 and light availability by hyponastic growth; shoot elongation; changes in leaf morphology and anatomy	*Rumex palustris* *Hordeum marinum* *Phalaris arundinacea*	Mommer *et al.* (2005b) Colmer and Pedersen (2008) Pedersen *et al.* (2010)
increased or maintained chlorophyll content	rice cultivars *Symmeria paniculata* *Tabernaemontana juruana*	Parolin (1997) Krack (2000) Sarkar *et al.* (2001)
less impairment in photosynthetic apparatus	flood-tolerant rice cultivars	Panda *et al.* (2008)
Growth (adverse impacts)		
no viability of seeds	many terrestrial plants	Hook (1984)
high mortality (50 – 100%) of seedlings, especially seedlings germinated in water	rice cultivars *Macrolobium acaciifolium* *Pterocarpus amazonum*	Ziburski (1990) Parolin (2002) Das *et al.* (2009) Oliveira-Wittmann (2006)
leaf senescence; chlorosis; necrosis; leaf abscission; poor survival	rice cultivars *Albizia multiflora* *Cecropia latiloba* *Crataeva benthamii* *Phragmites australis*	Mauchamp *et al.* (2001) Panda *et al.* (2008) Sarkar *et al.* (2006) Parolin (2009)
shoot growth cessation; thin and fragile shoots; declined shoot biomass	*Eleocharis cellulose*	Macek *et al.* (2006)
root growth cessation; cessation of biomass accumulation	Amazonian floodplain trees *Lotus tenuis*	Worbes (1997) Manzur *et al.* (2009)
impaired plant health and reduced growth rate; strongly decreased biomass production	azuki bean *Phragmites australis* *Lotus tenuis*	Ooume *et al.* (2009) Mauchamp *et al.* (2001) Schueler and Holland (2000) Manzur *et al.* (2009)
Growth (acclimation)		
fast seed germination upon desubmergence	some Amazonian floodplain trees	Koshikene (2005)
low seedling mortality (0 – 50%)	*Buchenavia oxycarpa* *Eschweilera ovalifolia* *Psidium acutangulum*	Ziburski (1990)

sprouting of new leaves from buds underwater and leaf expansion upon de-submergence; maintenance of existing leaves	*Cecropia latiloba* *Nectandra amazonum* *Psidium acutangulum*	Parolin (2009)
(wetland plants) hyponastic growth; increased specific leaf area; formation of gas films on the leaf surface; thinner epidermal cell wall; thinner cuticle; horizontal orientation of chloroplasts parallel to the epidermis; stomata with minimal or without cuticles	*Hordeum marinum* *Rumex palustris* *Mentha aquatic*	Mommer *et al.* (2005b) Pedersen *et al.* (2010)
(Amazonian floodplain trees) large epidermal cells; thick outer-epidermis walls; stomata on the adaxial leaf surface; sunken stomata on the abaxial leaf surface	*Eugenia inundata* *Garcinia brasiliensis* *Cassia leiandra* *Psidium acutangulum*	Parolin (2009)
pith cavity in shoots; enhanced shoot elongation; periodical growth reductions (judged by increment of tree rings)	rice cultivars some tropical trees *Gustavia augusta*	Worbes and Junk (1989) Parolin (2009) Vu *et al.* (2010)
formation of adventitious roots from leaf veins or stems; formation of schizogenous aerenchyma and large lysigenous intercellular spaces; suberized and lignified barriers to O_2 radicals; loss of exodermis in basal roots	rice cultivars *Garcinia brasiliensis* *Cassia leiandra* *Psidium acutangulum* *Salix martiana* *Nectandra amazonum* *Hordeum marinum*	Colmer (2003) De Simone *et al.* (2003) Oliveira-Wittmann (2006) Parolin (2009) Pedersen *et al.* (2010)
dormant during submergence; completing life cycle between submergence intervals	*Chenopodium ubrum*	Van Der Sman *et al.* (1992)

Carbohydrate catabolism	Enzymes	Species	References
Glycolysis (anoxia)			
sucrose ⟶ glucose + fructose starch ⟶ glucose glucose ⟶ glucose-6-P glucose-6-P ⟶ fructose-6-P	sucrose synthase α-amylase glucose kinase	rice seedlings maize seedlings	Ricard *et al.* (1991) Guglielminetti *et al.* (1995) Guglielminetti *et al.* (1997) Perata *et al.* (1997)
fructose-6-P ⟶ fructose-1,6-P_2 fructose-1,6-P_2 ⟶ pyruvate	phosphofructokinase pyruvate kinase	rice coleoptiles	Gibbs *et al.* (2000)
Fermentation (hypoxia or anoxia)			
pyruvate ⟶ acetaldehyde acetaldehyde ⟶ ethanol	pyruvate decarboxylase alcohol dehydrogenase	rice seedlings maize roots wheat roots red beet	Roberts *et al.* (1984) Johnson *et al.* (1994) Bouny and Saglio (1996) Waters *et al.* (1991) Zhang and Greenway (1995) Rivoal *et al.* (1989)
acetaldehyde ⟶ acetate	aldehyde dehydrogenase	maize leaves	Tsuji *et al.* (2003) Meguro *et al.* (2006)

mergence. In susceptible rice cultivars, submergence causes degradation of chlorophyll, decrease of Rubisco activity and damage to photosynthetic apparatus, resulting in strong reduction of photosynthesis (Ella *et al*., 2003; Panda *et al*., 2008). Anaerobic metabolic pathways induced under hypoxic or anoxic conditions (hypoxia, < 20.9% and > 0% O_2 at 20^oC; anoxia, 0% at 20^oC) are far less efficient in energy conversion than aerobic respiration, and thus they can deplete carbohydrate reserves more rapidly (Laan and Blom, 1990; Guglielminetti *et al.*, 1997). If the reserves of soluble sugars and starch are not replenished, exhaustion of carbohydrate will ultimately lead to cell and organ death (Bailey-Serres and Voesenek, 2008).

1.1.2 Energy crisis

Submergence causes carbohydrate shortage in plants (1.1.1), diminishing important substrates to sustain glycolysis and ATP generation. The majority of ATP for cellular metabolism in plants is generated by oxidative phosphorylation (i.e. through respiration) under aerobic conditions. Submergence often causes hypoxia within shoot tissues and can cause anoxia in non-photosynthesizing tissues such as roots (Armstrong *et al*., 1994; Mommer *et al*., 2004). Upon sensing hypoxia/anoxia, plants alter metabolism to generate ATP via glycolysis followed by fermentation (Fig. 1). Energy (ATP) crisis ensues because glycolysis is inefficient, yielding 2 to 3 mol ATP per mol hexose, as compared with 24 to 36 mol ATP generated by oxidative phosphorylation (Gibbs and Greenway, 2003). Although studies in different plant species have demonstrated that glycolysis and fermentation are necessary for cell survival under O_2 deprivation, enhancement of these processes is not necessarily correlated with prolonged submergence tolerance in plants (Gibbs and Greenway, 2003).

Low ATP availability leads to cellular damage, owing to deterioration of cellular components such as membranes (Gibbs and Greenway, 2003), and/or cytoplasmic acidosis in anoxia-sensitive species (Xia and Roberts, 1996). Furthermore, ATP-dependent processes such as DNA synthesis and cell division are curtailed (Gibbs and Greenway, 2003) and production of rRNA becomes dramatically reduced (Fennoy *et al*., 1998). Accordingly, low O_2 has been shown to markedly limit protein synthesis in *Arabidopsis* and maize while maintaining translation initiation of a subset of cellular mRNAs to lessen ATP expenditure (Fennoy *et al.*, 1998; Branco-Price *et al.*, 2005).

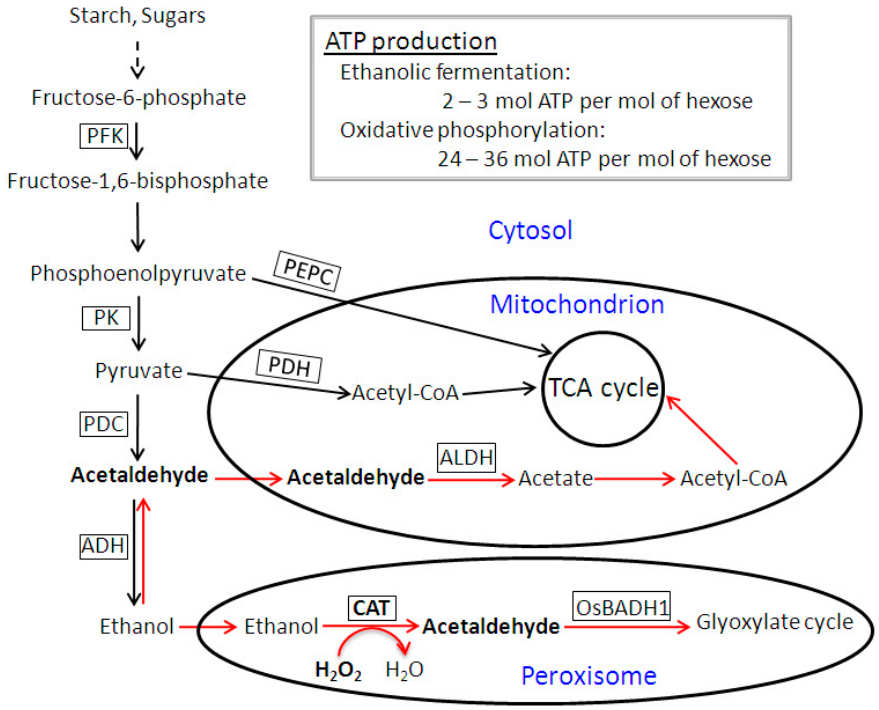

Fig. 1. Pathways of anaerobic carbohydrate catabolism showing key reactions in fermentation and detoxification of acetaldehyde.

Compounds and enzymes indicated in bold were analyzed in this study (chapter 6.3). Red lines indicate the pathways of acetaldehyde oxidation in mitochondria and peroxisomes proposed for rice during submergence and following re-aeration. Oxidative phosphorylation under aerobic conditions allows much higher ATP production per mol carbohydrate compared with ethanolic fermentation. Under submergence condition, pyruvate is converted to acetaldehyde by pyruvate decarboxylase (PDC). In parallel, acetaldehyde is converted to ethanol by alcohol dehydrogenase (ADH) and to acetate by acetaldehyde dehydrogenase (ALDH). When submerged plants become re-aerated, anaerobically accumulated ethanol is rapidly oxidized to acetaldehyde by the reverse reaction of ADH. Acetaldehyde can be transported into mitochondria and oxidized to acetate by ALDH. In peroxisomes, on the other hand, catalase (CAT) can oxidize ethanol to acetaldehyde during the conversion of H_2O_2 to H_2O. Subsequently, betaine aldehyde dehydrogenase (OsBADH1) is supposed to oxidize acetaldehyde to acetate in rice. Acetate is further converted to acetyl-CoA and used in glyoxylate cycle. Other abbreviations are: PDH, pyruvate dehydrogenase; PEPC, phosphenolpyruvate carboxylase; PFK, phosphofructokinase; PK, pyruvate kinase.

1.1.3 Toxicities of reduced soil components

In water-saturated conditions, electrochemical properties of soils also change because of the activities of microorganisms that use oxidized chemicals as electron acceptors (Ponnamperuma, 1984; Laanbroek, 1990). The concentrations of certain potentially toxic compounds, such as Mn^{2+}, Fe^{2+} and S^{2-}, often increase in flooded soil, which may lead to their accumulation in root tissues (Jackson and Drew, 1984). Moreover, volatile lower organic acids (e.g. propionic and butyric acids) can also accumulate in flooded soils and damage roots (Armstrong and Armstrong, 1999). These organic acids, together with high [CO_2], can impose "acid loads" on root cells in submerged plants (Greenway *et al*., 2006).

1.1.4 Mechanical damage

Plants can suffer physically from strong water flow or from abrasion by suspended particles during flooding (Jackson and Ram, 2003).

Growth and developmental processes in plants can be strongly disturbed and restricted under these adverse conditions during submergence (Tab. 1). For instance, seeds of most terrestrial plants lose viability under submergence stress (Hook, 1984). Seedlings, especially those germinated in water, have high mortality in flood-prone areas (Parolin, 2002; Das *et al.*, 2009). Above- and belowground growth and biomass accumulation become severely impaired, or even completely ceased, during submergence in many terrestrial plants (Mauchamp *et al.*, 2001; Macek *et al.*, 2006; Sarkar *et al.*, 2006; Parolin, 2009).

1.2 Stress after de-submergence

After de-submergence, tissue injuries which developed underwater can be intensified as the floodwater recedes and shoots become re-exposed to the atmosphere (Sarkar *et al*., 2006). The major forms of stress plants must encounter upon de-submergence are explained in the following sections.

1.2.1 Photoinhibition

The photosynthetic carbon fixation and accumulation of carbohydrate are essential for biomass accumulation during flood intervals, and further, for survival of future flooding. However, a sudden increase in light intensity upon de-submergence threatens leaves accustomed to low-light underwater environments, causing photoinhibition to the photosynthetic apparatus (Osmond, 1994; Ella *et al.*, 2003). For example, submergence increased minimal fluorescence in leaves of rice cultivars, which signifies photoinhibition (Panda *et al*., 2006). The first challenges for de-submerged plants thus include photoprotection and recovery from photoinhibition.

1.2.2 Production of reactive oxygen species (ROS)

The main cellular components susceptible to damage by free radicals are membrane lipids (peroxidation of unsaturated fatty acids), proteins (denaturation), carbohydrate and nucleic acids (Blokhina *et al*., 2003). ROS can be generated under hypoxic conditions during submergence; severe lipid peroxidation by ROS can have a fatal consequence in submerged plants (Santosa *et al*., 2007). Hence, maintenance of membrane integrity is one of the key factors for plant survival underwater (Blokhina *et al*., 2003). Since *de novo* lipid synthesis requires energy and carbon, preservation of membrane lipids is a more economic way to maintain cell integrity in submerged plants. Anoxia-tolerant plant species, such as *Acorus calamus* and *Schoenoplectus lacustris*, are able to preserve lipids during submergence and after de-submergence, while significant lipid peroxidation happens in anoxia-sensitive plants, such as *Iris germanica*, upon re-oxygenation (Henzi and Braendle, 1993). In fact, production of ROS is far more intensified upon de-submergence, which is associated with an abrupt and concomitant increase in light intensity (1.2.1) and O_2 concentration. Excessive formation of ROS is commonly found in plants re-exposed to the ambient conditions following submergence (Blokhina *et al.*, 2003; Bailey-Serres and Chang, 2005; Santosa *et al.*, 2007). On the other hand, some ROS and oxidation products can serve as important signaling molecules in plant cells (Bailey-Serres and Chang, 2005; Moller *et al*., 2007).

Of the ROS species, superoxide (O_2^-), hydrogen peroxide (H_2O_2) and highly reactive hydroxyl radical (OH^*) can be produced in a number of cellular reactions: through non-enzymatic generation via electron transport chains in chloroplasts and mitochondria, through

iron-catalyzed Fenton reactions, and through various enzymatic reactions, such as those by lipoxygenase, NADP oxidase and xanthine oxidase (Mittler, 2002; Blokhina *et al.*, 2003; Apel and Hirt, 2004). In chloroplasts, excessive excitation of chlorophylls can result in formation of singlet O_2 ($^1O_2$) which initiates lipid peroxidation in photosynthetic membranes (Mittler, 2002).

1.2.3 Accumulation of acetaldehyde

Over-accumulation of acetaldehyde is harmful for organisms because of its tendency to form acetaldehyde-protein and acetaldehyde-DNA adducts. Acetaldehyde can be produced enzymatically from pyruvate or ethanol under both anoxic and hypoxic conditions, as shown in Fig. 1, with the levels produced during hypoxia being higher than anoxia (Boamfa *et al*., 2005). Re-aeration can induce a transient burst of acetaldehyde emission (Zuckermann *et al.*, 1997; Tsuji *et al.*, 2003) as a result of rapid ethanol oxidation without coordinated oxidation of acetaldehyde (Boamfa *et al*., 2005). In addition, detoxification of H_2O_2 by CAT can also produce acetaldehyde from ethanol (Perata *et al.*, 1992; Zhang *et al.*, 1997; Boamfa *et al.*, 2005). Thus, H_2O_2 formed under oxidative conditions can lead to lipid peroxidation (1.2.2), or alternatively, acetaldehyde accumulation in cells containing ethanol.

1.2.4 Drought

When the floodwater recedes, low hydraulic conductivity of submerged roots cannot provide enough water to meet aboveground transpirational demand, causing wilting of shoot organs in a range of plant species (Holbrook and Zwieniecki, 2003).

1.3 Acclimation to submergence

Submergence-tolerant species are able to alleviate adverse impacts of submergence and de-submergence by changing the morphology and anatomy of shoots and roots, adjusting metabolic pathways, or upregulating antioxidant defense mechanisms. These acclimatory responses, which are expressed mainly by submergence-tolerant plants, are described below (see also Tab. 1).

1.3.1 Anatomical and morphological acclimation

Certain morphological responses, such as increased specific leaf area, vertical leaf orientation or petiole elongation, are induced by both shade and submergence stress; however, acclimation to submergence is directed predominantly to an improved capacity for gas exchange rather than light capture (Mommer *et al*., 2005a). Altered morphology and anatomy of submerged shoots and roots can facilitate underwater gas exchange in leaves and internal gas diffusion between leaves and roots (Evans, 2004; Mommer *et al.*, 2005b; Colmer and Pedersen, 2008; Jung *et al.*, 2008).

Leaf morphology and anatomy Diffusion across the cuticle may be the major pathway for O_2 and CO_2 entry into leaves underwater (Frost-Christensen *et al*., 2003), even for leaves with stomata (i.e. aerial leaves of amphibious species and leaves of wetland plants). In order to enhance gas exchange with the surrounding floodwater, some plants produce aquatic leaves during submergence (Mommer and Visser, 2005). For example, submergence-acclimated leaf blades of *Rumex palustris* have thinner epidermal cell walls and a thinner cuticle, and chloroplasts in mesophyll cells are oriented towards epidermis (Mommer *et al*., 2005b). Enhanced CO_2 uptake via aquatic leaves promotes underwater photosynthesis which supplies carbohydrate as well as O_2 to submerged plants. Consequently, the O_2 status is improved in shoots not only by photosynthetic O_2 evolution in aquatic leaves during the day, but also by better entry of O_2 in these leaves from the water column during the night (Mommer and Visser, 2005). Through internal O_2 diffusion in aerenchyma, roots also benefit from the improved O_2 status in shoots (Jackson and Armstrong, 1999).

Hyponastic growth and shoot elongation Hyponastic growth refers to upward growth of plant organs (usually leaves and shoots) caused by differential cell elongation between the upper and lower surfaces of the organ (Banga *et al*., 1997). By hyponastic growth submerged plants of some species can decrease the distance between the leaf/shoot tips and the water surface. Several wetland plants, such as *Rumex* species and rice, exhibit stimulated hyponastic growth (elongation) of petioles and shoot internodes when submerged completely (Ridge, 1987). Shoot elongation depends largely on cell elongation (Voesenek *et al*., 1990) which involves loosening of the otherwise rigid cell walls (Cosgrove, 2005). Cell elongation also requires synthesis of new cell walls, and hence the availability of energy and carbohydrate.

Lowland rice cultivars, which respond to submergence by elongation growth, have lower survival rates due to higher energy consumption (Setter and Laureles, 1996).

Aerenchyma and pith cavity formation Aerenchyma is tissue with large gas-filled spaces (lacunae) that interconnect longitudinally to provide a low-resistance pathway for long-distance gas transport along plant organs. In stems and rhizomes aerenchyma can form in the cortex and in the pith cavity, whereas in roots it is usually found in the cortex (Armstrong, 1979). Two types of aerenchyma formation are known (Sachs, 1882): by separation ("schizogenous" aerenchyma) or collapse of cells ("lysigenous" aerenchyma). In addition to these two main forms, several other processes can also form aerenchyma (Jung *et al*., 2008). Many wetland plants show constitutive formation of aerenchyma, which is further enhanced in response to flooding. Ethylene is the primary signal that initiates programmed cell death in the cortical cells to form lysigenous aerenchyma (Drew *et al*., 2000).

1.3.2 Physiological acclimation

Acclimation to submergence is achieved not only by anatomical and morphological changes, but also by metabolic adjustment in response to O_2 deficiency. Moreover, some metabolic responses are involved in inducing morphological/anatomical changes.

Hormone signaling Ethylene is considered to be a reliable indicator for complete submergence in green tissues (Voesenek *et al*., 2006). The interplay of ethylene, abscisic acid (ABA) and gibberellin (GA) regulates shoot elongation (1.3.1) in submerged plants. Ethylene accumulation reduces ABA levels by inhibiting ABA biosynthesis (Benschop *et al*., 2005) and at the same time increasing ABA degradation (Saika *et al*., 2007). This decline of ABA, in turn, releases repression of GA biosynthesis and thus increases the GA concentration in cells, leading to shoot elongation (Benschop *et al*., 2006). Flooding-induced elongation growth and carbohydrate catabolism are regulated by ethylene-responsive transcription factors in rice; *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*) genes stimulate elongation growth in leaves and stems of deepwater cultivars (Hattori *et al*., 2009), whereas *SUBMERGENCE1A-1* (*SUB1A-1*) gene suppresses elongation growth and carbohydrate catabolism in rice cultivars (Fukao *et al*., 2006; Xu *et al*., 2006). The *SK* genes act upstream of GA, but it is not yet known whether they interact with GA (Voesenek and Bailey-Serres, 2009). While triggering these acclimatory

responses during submergence, ethylene accumulation can decrease chlorophyll content and antioxidant capacities in leaves after de-submergence (Kawano *et al.*, 2002; Ella *et al.*, 2003).

Carbohydrate catabolism Previous studies with submergence-tolerant and -intolerant rice cultivars indicated that seedlings of the tolerant cultivars have 30 – 50% more non-structural carbohydrate compared to the susceptible cultivars (Chauturvedi *et al.*, 1995; Setter *et al.*, 1997; Sarkar, 1998; Sarkar *et al.*, 2006). Non-structural carbohydrate is utilized by plants as the major energy source for maintenance metabolism during submergence.

Responses of carbohydrate catabolism to anaerobic conditions are listed in Table 1. The metabolic responses to O_2 deficiency are often orchestrated by the availability and mobilization of carbohydrate. Anoxia-tolerant plants, like rice, *Acorus calamus* and *Potamogeton pectinatus*, are able to degrade starch under low O_2 conditions by, for example, increasing the activity of α-amylase (Arpagaus and Braendle, 2000; Dixon *et al*., 2006; Fukao *et al*., 2006; Magneschi and Perata, 2009). Sucrose, which is the major transport form of carbohydrate in rice and many other plants, can be hydrolyzed in two distinct ways: by sucrose synthase and invertase. The net cost for entry into glycolysis is 1 mol pyrophosphate (PPi) per mol sucrose by energy-efficient sucrose synthase; in comparison, invertase is less energy-efficient and the cost is 2 mol ATP per mol sucrose (Bailey-Serres and Voesenek, 2008). It has been shown that the transcription and the enzymatic activity of sucrose synthase increase, whereas those of invertase decrease, during O_2 deprivation in deepwater rice, maize roots and potato tubers, suggesting that sucrose synthase is the principal enzyme for conversion of sucrose to phosphorylated hexose sugars under O_2 deficiency (Zeng *et al.*, 1999; Geigenberger, 2003; Fukao *et al.*, 2006). Hexose sugars then enter glycolysis and are finally converted to ethanol, the end-product of anaerobic carbohydrate catabolism, by pyruvate decarboxylase and alcohol dehydrogenase (Fig. 1).

C_3/C_4 switch An amphibious plant, *Eleocharis vivipara*, can switch between the C_4 and C_3 mode of photosynthesis under terrestrial and submerged conditions, respectively (Ueno *et al.*, 1988). In terrestrial conditions, photosynthetic culms of *E. vivipara* possess a Kranz-type anatomy typical of C_4 plants and their well-developed bundle-sheath cells contain numerous large chloroplasts. When underwater, *E. vivipara* develops anatomical features of aquatic plants and the bundle-sheath cells are reduced and contain only a few small chloroplasts. The C_4 pathway operating under terrestrial conditions produces aspartate and malate as the primary

products, as in the NAD-malic enzyme C_4 pathway. The C_3 pathway in submerged culms, having increased activities of ribulose 1,5-bisphosphate carboxylase (Rubisco), supplies 3-phosphoglyceric acid and sugar phosphates as the main primary products (Ueno *et al*., 1988).

Antioxidant defense systems Chloroplasts and peroxisomes are major organelles of ROS generation in green tissues while it is mitochondria in non-green tissues. To prevent or minimize damage caused by ROS, plant cells are equipped with antioxidant defense systems, consisting of antioxidants such as ascorbate, α-tocopherol, glutathione and carotenoids as well as antioxidant enzymes (Mittler, 2002; Blokhina *et al*., 2003). The major pathways of ROS scavenging in chloroplasts, peroxisomes and mitochondria of plant cells are illustrated in Figure 2. Peroxidation reactions triggered by ROS can be terminated by the action of superoxide dismutase (SOD), the major scavenger of superoxide anion (O_2^-) in almost all cellular compartments (Meloni *et al*., 2003). H_2O_2 is then detoxified by ascorbate peroxidase (APX) in the water-water cycle (chloroplasts), by ascorbate-glutathione cycle (chloroplasts, cytosol, mitochondria, apoplast and peroxisomes), or by catalase in peroxisomes (Mittler, 2002). Contrasting responses of antioxidant systems in leaves and roots have been reported in some species during different abiotic stress as well as subsequent recovery (Cavalcanti *et al*., 2006, Hossain *et al*., 2009, Skutnik and Rychter, 2009). C_4 plants such as maize, reed, *Egeria densa*, *Anabasis articulata* are characterized by effective antioxidant defense which, together with the C_4 pathway, confers environmental stress tolerance (Mittler *et al.*, 2001; Casati *et al.*, 2002; Stepien and Klobus, 2005).

As shown in Figure 1, plants have also mechanisms to detoxify acetaldehyde; in cytosol and mitochondria aldehyde dehydrogenase can convert acetaldehyde to far less toxic acetate (Meguro *et al.*, 2006, Fujiwara *et al.*, 2008). In peroxisomes of rice seedlings, a betaine aldehyde dehydrogenase (OsBADH1) oxidizes acetaldehyde which is produced from ethanol by CAT activities (Mitsuya *et al*., 2009).

1.4 Strategies for submergence tolerance

Some of the acclimatory responses of terrestrial plants to submergence described above (1.3) can be attributed to two contrasting strategies of flood tolerance: the “escape” and “quiescence” strategies (Perata and Voesenek, 2007, Bailey-Serres and Voesenek, 2008). Plants with the

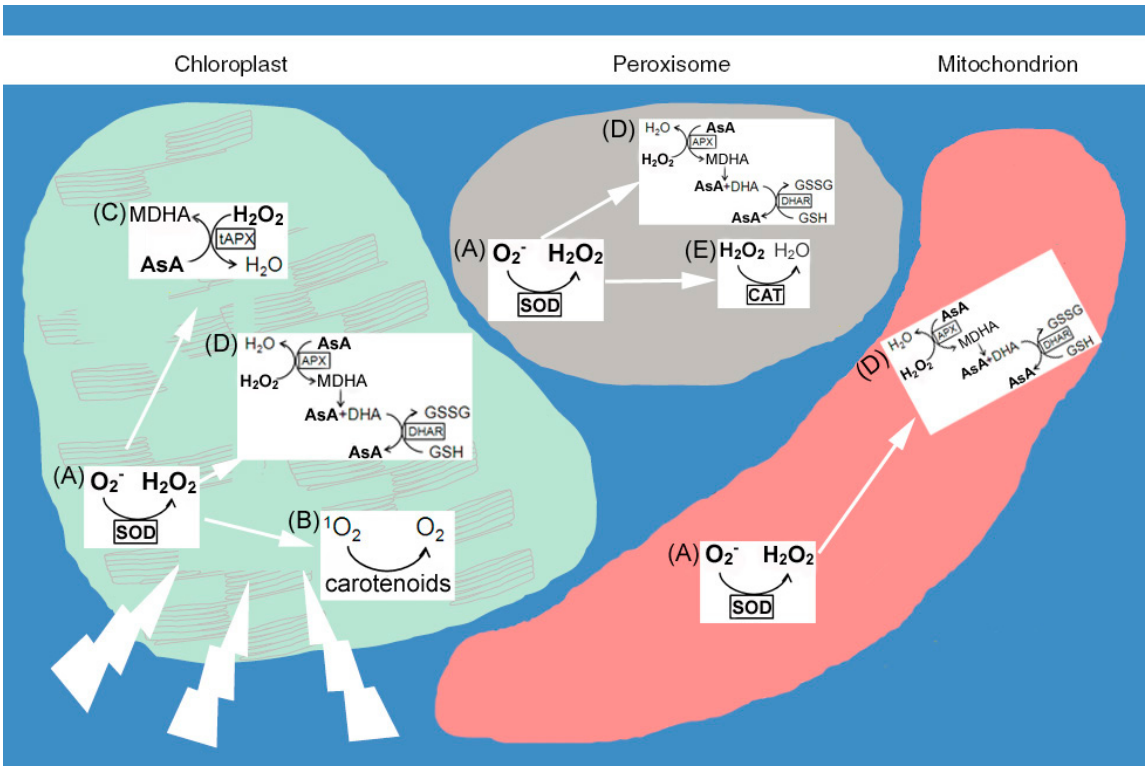

Fig. 2. The major pathways for scavenging of reactive oxygen species (ROS) in chloroplasts, peroxisomes and mitochondria of plant cells.

Compounds and enzymes indicated in bold were analyzed in this study (chapter 6.3). Superoxide dismutase (SOD) catalyzes the reaction from superoxide (O_2^-) to hydrogen peroxide (H_2O_2) in different cellular compartments (A). Carotenoids (B) and the water-water cycle (A→C) are found in chloroplasts. The ascorbate-glutathione cycle operates in chloroplasts, mitochondria and peroxisomes (D). Catalase (CAT) is located in perxisomes (E). Abbreviations are: APX, ascorbate peroxidase; AsA, reduced ascorbate; DHA, dehydroascorbate; DHAR, DHA reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; MDHA, monodehydroascorbate; MDAR, MDHA reductase; $^1O_2$, singlet excited oxygen; tAPX, thylakoid-bound APX.

escape strategy respond to submergence by enhancing shoot elongation to regain contact with the atmosphere, whereas those with the quiescence strategy conserve energy and carbohydrate by restraining growth. The costly escape strategy is advantageous in shallow floodwater, in which elongation growth allows plants to re-establish air contact; failure to do so results in accelerated depletion of carbohydrate reserves. The quiescence strategy, on the other hand, can save energy and resource during submergence, which could positively affect the survival

rate and generation of new tissues after de-submergence (Das *et al*., 2005; Fukao *et al*., 2006; Panda *et al*., 2008), provided that the flooding period is not too long.

2. Motivation

The capacity for fast growth recovery after de-submergence, withstanding the weakness and injury from submergence and the difficulties in re-acclimating to terrestrial conditions, is crucial for successful establishment of riparian species in water-level-fluctuation zones. While our understanding of the mechanisms of flooding survival in plants has been advanced remarkably in recent years (Gibbs and Greenway, 2003; Bailey-Serres and Voesenek 2008), few studies were focused on recovery processes and responses that happen in plants after de-submergence. For example, much less is known about post-submergence recovery of photosynthesis and growth in flood-tolerant plants, even though these carbon acquisition and accumulation processes are essential for species performance during flood intervals, and further, for survival of future flooding. Thus, in this study I compared the recovery behavior of two wetland species, *Alternanthera philoxeroides* and *Hemarthria altissima*, which are naturally distributed at low elevations of the water-level-fluctuation zones of the Three Gorges Reservoir region in China. In an attempt to infer the strategies of submergence tolerance in these two species, I first investigated submergence-induced morphological and anatomical responses in these plants. Following de-submergence, interactions between antioxidant defense, photosynthesis, carbohydrate partitioning and growth were closely examined both in shoots and roots. Low-light stress, instead of submergence stress, was additionally applied to some plants to identify the responses and effects which are specifically induced by submergence.

3. Discussion

Flood-tolerant species can grow at low elevations along the floodplain while less flood-tolerant species are confined to higher elevations of the floodplain gradient (van Eck *et al.*, 2004). The two riparian species examined in this study, *A. philoxeroides* and *H. altissima*, occur at low-elevation sites of river banks and are capable of enduring long-term submergence. However, different survival rates, 50% and 90% for *A. philoxeroides* and *H. altissima*, respectively, have been reported after 180 d of complete submergence (Wang *et al.*, 2008a, b). The better survival of *H. altissima* than *A. philoxeroides* after long-term submergence may be explained by different acclimatory responses of these two plants during submergence and de-submergence.

Submergence enhanced stem elongation in *A. philoxeroides* (Fig. 4A, B in Luo *et al*., 2009), a morphological response observed in certain flood-tolerant species (1.3.1) (Mommer *et al.*, 2005b; Jackson, 2008). Rapid stem elongation serves to regain a contact with the atmosphere or well-oxygenated upper water layers. Once contact with the atmosphere is established in relatively shallow water, these plants can perform efficient photosynthesis and survive prolonged submergence (Voesenek *et al.*, 2004). Strongly elongated stems and petioles are also found in plants under shade environments ("shade avoidance"; Ballaré *et al.*, 1999; Valladares, 2003; Franklin and Whitelam, 2005). However, plants of *A. phioloxeroides* in the shading treatment did not exhibit enhanced stem elongation in this study (Fig. 4A, B in Luo *et al*., 2009). Thus, it seems that the elongation response of *A. phioloxeroides* was induced specifically by submergence, not by shading, probably via ethylene signalling (1.3.2) (Benschop *et al.*, 2005; Mommer *et al.*, 2005a; Cox *et al.*, 2006; Jackson, 2008).

In contrast, plants of *H. altissima* did not elongate internodes in response to submergence (Fig. 4C, D in Luo *et al*., 2009). Instead of trying to escape, this plant may have adopted a "quiescence" strategy (1.4) to survive submergence (Geigenberger, 2003; Voesenek *et al.*, 2006).

3.1 Post-submergence recovery of *A. philoxeroides*, showing escape strategy

After 20 d of complete submergence, the carbohydrate storage of leaf, stem and root tissues was largely depleted in *A. phioloxeroides* (Figs 6 – 8 in Luo *et al*., 2010). Under energy crisis,

such as during the 20-d shading and submergence treatments applied in this study, remobilization of stored carbohydrate plays a vital role in providing energy needed for cell and organ maintenance as well as stress responses (1.3.2) (Greenway and Gibbs, 2003). The submergence-induced stem elongation, even if it involved little dry mass accumulation (Fig. 5B in Luo *et al*., 2010), may have contributed to carbohydrate depletion in the submerged plants of *A. philoxeroides*, as was seen in *Rumex palustris* showing submergence-induced petiole elongation (Groeneveld and Voesenek, 2003).

Following 20 d of shading, carbohydrate accumulation immediately recovered in the de-shaded plants of *A. philoxeroides* on day 21 (Figs 6 – 8 in Luo *et al*., 2010). The slightly lower maximal photosystem II (PSII) efficiency (Fv/Fm) of the de-submerged plants early in the recovery period (Fig. 2A in Luo *et al*., 2010), presumably caused by structural and/or functional damages to chloroplasts (Panda *et al.*, 2006), may have delayed the recovery of carbohydrate accumulation in these plants. With their carbohydrate storage nearly exhausted (Figs 6 – 8 in Luo *et al*., 2010), carbohydrate and biomass accumulation of the de-shaded and de-submerged plants of *A. philoxeroides* must have been largely dependent on quick recovery of photosynthesis.

The ability for flexible photosynthetic acclimation may be essential for *A. philoxeroides* living in fluctuating water, in which the availability of light, CO_2 and O_2 changes dramatically and periodically (Luo *et al.*, 2009). The dynamic adjustment of photosynthesis and photoprotective thermal energy dissipation (Figs 5 – 7 in Luo *et al*., 2009) was accompanied by similarly dynamic antioxidative defense responses in leaves of *A. philoxeroides* (Figs 7 – 9 in Luo *et al*., 2010 submitted); all three antioxidants examined, SOD, CAT and AsA, were downregulated during submergence but recovered within 3 – 6 d after de-submergence. Likewise, the levels of O_2^- and H_2O_2 were quickly normalized in a few days of recovery (Figs 2 and 4 in Luo *et al*., 2010 submitted). Despite dramatic changes in light and O_2 levels upon de-submergence, the extent of lipid peroxidation was rather small in this species (Fig. 6A in Luo *et al*., 2010 submitted). Also, acetaldehyde increased in leaves and roots only transiently after de-submergence (Fig. 1A and C in Luo *et al.*, 2010 submitted). These results indicate the ability of *A. philoxeroides* to quickly upregulate detoxification pathways and protective mechanisms against (photo-) oxidative damage to achieve rapid recovery of photosynthesis and growth.

Recovery of leaf and root growth started 1 or 2 d earlier in the de-shaded plants than in the de-submerged plants for *A. philoxeroides* (Figs 3 and 4A, E in Luo *et al*., 2010). The faster growth recovery of the de-shaded plants is consistent with the high Fv/Fm (Fig. 2A in Luo *et al*., 2010) and the immediate recovery of carbohydrate accumulation found in their leaves on day 21 (Figs 6 – 8 in Luo *et al*., 2010). The somewhat reduced photosynthetic capacity of the de-submerged plants early in the recovery period may have delayed the recovery of carbohydrate accumulation and growth in these plants. Indeed, the strong leaf growth recovery on day 23 coincided with the full recovery of Fv/Fm in the de-submerged plants (Figs 2A and 3A in Luo *et al*., 2010), demonstrating parallel recovery of photosynthesis and growth in *A. philoxeroides*. Shortly after de-submergence, the relative dry mass increase was the highest in leaves (Fig. 5A in Luo *et al*., 2010), the primary organ for photosynthesis, and the lowest in roots. Similarly, the onset of leaf growth recovery preceded that of root growth recovery by 1 – 2 d (Figs 3A and 4A in Luo *et al*., 2010). Together, these results suggest that restoration of photosynthesis and photosynthetic organs has high priority in carbohydrate-depleted, de-submerged plants of *A. philoxeroides* during the initial recovery period.

3.2 Post-submergence recovery of *H. altissima*, showing quiescence strategy

Unlike *A. philoxeroides*, plants of *H. altissima* retained ≥ 10 μmol sucrose g^{-1} fresh weight in leaves and culms after 20-d submergence (Figs 6F and 7F in Luo *et al*., 2010). Since the light intensity in the submergence treatment was extremely low (less than 10 μmol photons m^{-2} s^{-1}), this sucrose accumulation in leaves and culms of *H. altissima* must have engaged synthesis of sucrose from carbohydrate remobilization, such as starch degradation. Anoxia-tolerant plants, like rice, *Acorus calamus* and *Potamogeton pectinatus*, are able to degrade starch under low O_2 conditions by, for example, increasing the activity of α-amylase (Arpagaus and Braendle, 2000; Dixon *et al.*, 2006; Magneschi and Perata, 2009). The peculiar accumulation of sucrose found in the submerged plants of *H. altissima* is consistent with the quiescence strategy (Luo *et al.*, 2009), showing no obvious growth response during submergence (Bailey-Serres and Voesenek, 2008). It seems that submergence downregulated energy consumption and carbohydrate catabolism in *H. altissima* (Gibbs and Greenway, 2003; Greenway and Gibbs, 2003), leading to the sucrose retention in the submerged plants, but not in the shaded plants (Figs 6 – 8 in Luo *et al*., 2010).

A sudden increase in light and O_2 upon de-submergence did not cause excessive formation of ROS in leaves of *H. altissima*; O_2^- and H_2O_2 could not be detected under all conditions (Figs 3 and 5 in Luo *et al*., 2010 submitted). Although de-submergence did increase acetaldehyde accumulation and lipid peroxidation in leaves (Figs 1B and 6B in Luo *et al*., 2010 submitted), the content of lipid peroxidation products gradually declined to the normal level during 10 d of recovery. The relatively high antioxidant capacities found in leaves of *H. altissima*, especially SOD and AsA (Figs 7B and 9B in Luo *et al*., 2010 submitted), may have contributed to the suppression of lipid peroxidation in leaves after de-submergence. Efficient antioxidant activities (Figs 7 – 9 in Luo *et al.*, 2010 submitted) would be essential in leaves of *H. altissima* having limited PSII efficiency in the light (Fig. 6 in Luo *et al.*, 2009); the capacity of PSII electron transport was always rather small in this plant, much smaller than that of *A. philoxeroides*. In fact, sudden increase in light intensity on day 20 resulted in significant decrease of the maximal PSII efficiency in both de-shaded and de-submerged plants of *H. altissima* (Fig. 2B in Luo *et al*., 2010), indicating the vulnerability of this species to photoinhibition (Luo *et al.*, 2009).

Sucrose stored in leaves and culms at the end of the submergence treatment was rapidly hydrolysed shortly after de-submergence (Figs 6F and 7F in Luo *et al*., 2010) and growth recovery started earlier than the full recovery of Fv/Fm in the de-submerged plants of *H. altissima* (Figs 2B, 3B and 4E in Luo *et al*., 2010). The rapid hydrolysis of sucrose stored in leaves and culms apparently led to growth promotion. The de-shaded plants of *H. altissima*, on the other hand, showed neither sucrose retention at the end of the shading treatment (Figs 6 – 8 in Luo *et al*., 2010) nor strong leaf growth recovery after de-shading (Fig. 3B in Luo *et al*., 2010). Besides the high survival rate after a long-term submergence (Wang *et al.*, 2008), this ability to achieve rapid growth recovery points to a benefit of the quiescence strategy, namely fast generation of new tissues after de-submergence by using the carbohydrate pool retained during submergence (Kawano *et al.*, 2002; Das *et al.*, 2005; Fukao *et al.*, 2006; Panda *et al.*, 2008; Striker *et al.*, 2008). In contrast to *A. philoxeroides*, the largest dry mass increase was always found in culms (Fig. 5E in Luo *et al*., 2010), a storage organ of *H. altissima*. Conservative biomass allocation to storage organs can confer stress tolerance to plants, such as under low irradiance or herbivory attack (Myers and Kitajima, 2007; Imaji and Seiwa, 2010). The results from *H. altissima* suggest that conservative biomass allocation to storage organs

during flood intervals, along with conservative carbohydrate consumption during flooding, is a part of the submergence tolerance based on the quiescence strategy.

3.3 What are the crucial traits of the escape and quiescence strategies?

The escape strategy, involving underwater elongation growth (Fig. 4 in Luo *et al.*, 2009), is costly and will only be selected for in environments where the costs are outweighed by benefits, such as improved O_2 and carbohydrate status (Bailey-Serres and Voesenek, 2008). This costly strategy is advantageous in shallow floodwater, in which elongation growth allows plants to regain contact with the atmosphere (Colmer, 2003; Evans, 2004; Jung *et al.*, 2008). When leaves emerge above the water surface, high porosity in shoots and roots (Fig. 1 in Luo *et al*., 2009) allow internal gas diffusion and aerobic respiration in roots and rhizomes (Pierik *et al.*, 2009). Low porosity suppresses root growth, nutrient uptake and root-dependent process of the shoot. Another important advantage of leaf emergence is improved supply of carbohydrate by photosynthesis (Pierik *et al.*, 2009), provided that leaves re-emerging from the submerged environment are capable of fast acclimation of photosynthesis and photo-protection (Figs 5 – 7 in Luo *et al*., 2009) as well as efficient antioxidative defense (Figs 7 – 9 in Luo *et al.*, 2010 submitted).

The porous shoots formed during submergence are rather weak and fragile, need to be strengthened or renewed as the floodwater recedes and plants return to terrestrial conditions. The low root hydraulic conductivity of de-submerged plants also needs to be improved to support high transpiration rates of their leaves having thin cuticles (Holbrook and Zwieniecki, 2003). Under carbohydrate deficiency following long flooding, plants must quickly recover photosynthesis (Luo *et al.*, 2009) to acquire energy needed to strengthen or renew these organs suffering from deterioration or injuries during submergence and shortly after de-submergence. Since sudden increase in light and O_2 can intensify oxidative injuries upon de-submergence, plants must have efficient antioxidative and detoxification mechanisms (Luo *et al*., 2010 submitted) to cope with excessive formation of ROS and accumulation of toxic oxidative products in different cells and organs (Sarkar *et al.*, 2001; Blokhina *et al.*, 2003; Santosa *et al.*, 2007). Finally, flexible resource allocation (Luo *et al.*, 2009, 2010) to organs for acquisition of most limiting resources, both during submergence (stems to gain light, O_2 and CO_2) and after

de-submergence (leaves to gain carbohydrate), may enable survival and success of plants with the escape strategy.

When the floodwater is deep and/or not permanent, plants having the quiescence strategy can outperform those with the escape strategy. The quiescence strategy is characterized by limited underwater growth (Fig. 4 in Luo *et al.*, 2009) and conservation of energy and carbohydrate (Fig. 6 in Luo *et al.*, 2010; Perata and Voesenek, 2007). Because complete submergence is often associated with severe limitation of light, plants with the quiescence strategy need to be shade-tolerant (Luo *et al.*, 2009). Low-O_2 conditions (0.1 – 0.75% O_2) can favor generation of ROS in cells (Santosa *et al.*, 2007). This can be avoided by inward diffusion of O_2 from the atmosphere or well-oxygenated upper layers of water (Pierik *et al.*, 2009). However, the quiescence strategy does not allow access to the atmosphere or upper water layers. Therefore, maintenance of high antioxidative capacities (Luo *et al*., 2010 submitted) is necessary for these plants even under low-O_2 conditions during submergence; failure to do so leads to peroxidation of lipid membranes (Santosa *et al.*, 2007).

After de-submergence, hydrolyzation of retained carbohydrate (Fig. 6 in Luo *et al.*, 2010) enables rapid growth recovery in leaves and roots even before the recovery of photosynthesis (Figs 2 – 4 in Luo *et al.*, 2010). Unlike the escape strategy, plants with the quiescence strategy must ensure the availability of sufficient carbohydrate storage to endure complete submergence. Conservative biomass allocation to storage organs during flood intervals (Fig. 5 in Luo *et al.*, 2010) is thus crucial for submergence tolerance based on the quiescence strategy.

In sum, the escape strategy requires porous structure of shoots and roots and stimulated shoot elongation during submergence. Shortly after de-submergence, quick adjustment of photosynthesis and antioxidative defense is essential for the escape strategy based on dynamic growth and biomass investment in organs which directly allow alleviation of resource limitations. In contrast, the quiescence strategy must conserve energy and carbohydrate by restricting growth underwater. Furthermore, to cope with low irradiance and utilize carbohydrate economically, while keeping high antioxidative capacities, is crucial to maintain the cell integrity and survive complete submergence with the quiescence strategy. De-submergence-induced hydrolysis of retained carbohydrate enables rapid start of re-growth, and conservative biomass allocation to storage organs increases the survival chance of the quiescence-strategy plants in the next flooding event.

4. Synopsis and Outlook

In this study, I compared the recovery processes of two wetland plants, *Alternanthera philoxeroides* and *Hemarthria altissima*, showing the escape and quiescence responses to submergence, respectively. Plants of *A. philoxeroides* quickly recovered the capacities for photosynthesis and antioxidative defense shortly after de-submergence, while *H. altissima* maintained relatively low photosynthetic capacities and high antioxidant activities (Figs 5 – 7 in Luo *et al*., 2009; 7 – 9 in Luo *et al*., 2010 submitted). It needs to be tested in further species whether these contrasting responses of photosynthesis and antioxidants are common traits among plants with the escape and quiescence strategies. Also, the question of how O_2^- could accumulate to detectable levels in apparently healthy leaves of *A. philoxeroides* demands an answer; despite high SOD activities and also high AsA contents, O_2^- was always detected in leaves of this plant under non-stressful growth conditions (Figs 2, 7 and 9 in Luo *et al*., 2010 submitted).

Growth recovery of the de-submerged plants of *A. philoxeroides* was largely dependent on newly synthesized photoassimilates; in contrast, growth recovery started earlier than the full recovery of Fv/Fm in the de-submerged plants of *H. altissima*, probably supported by rapid hydrolysis of sucrose stored in shoots (Figs 2 – 4, 6 – 8 in Luo *et al*., 2010). The distinct coordination and interactions between photosynthesis, carbohydrate accumulation and growth by the escape and quiescence strategies following de-submergence could be clearly demonstrated in the two species in the present study. The processes and mechanisms underlying these contrasting recovery patterns are yet to be explored. For example, are newly synthesized photoassimilates and carbohydrate hydrolyzed from stored sucrose used in the same or different processes in *H. altissima* during recovery shortly after de-submergence? Which enzyme (sucrose synthase or invertase) catalyzes the hydrolysis of sucrose in *H. altissima* and how is the enzyme activated upon de-submergence?

Compared with leaf growth, recovery of root growth was delayed by 1 – 2 d in the de-submerged plants of both species (Figs 3 and 4A, E in Luo *et al*., 2010), suggesting that the newly synthesized carbohydrate is initially retained in leaves and used for shoot growth. Poor sink strength of roots after severe O_2 and energy limitation during submergence (Gaynard and Armstrong, 1987; Crawford, 1992; Armstrong *et al.*, 1994) may also contribute to the slower growth recovery of roots. During the initial recovery, biomass allocation was directed most

strongly to leaves in *A. philoxeroides* (Fig. 5A – C in Luo *et al*., 2010), whereas *H. altissima* always invested the largest biomass in culms (Fig. 5D – F in Luo *et al*., 2010). Control mechanisms of sink strength in different organs and regulation of biomass allocation in *A. philoxeroides* and *H. altissima* during recovery deserve investigations.

A. philoxeroides and *H. altissima* are a C_3 herb and a C_4 grass, respectively, which are naturally distributed in the water-level-fluctuation zone of the Three Gorges Reservoir area in China. Many other flood-tolerant species having the C_4 pathway (Wang *et al.*, 2008a, b), such as *Arundinella anomala*, *Cynodon dactylon*, *Paspalum distichum*, *Vetiveria zizanioides*, can be found in this flood-prone area. The C_4 pathway is regarded as superior to the C_3 pathway under stress conditions, mainly because of its high water use efficiency based on the carbon concentration mechanism (Hatch, 1992; Furbank and Taylor, 1995; Ku *et al.*, 1996; Mittler *et al.*, 2001). High antioxidant activities found in leaves of *H. altissima* in this study (Luo *et al.*, 2010 submitted) may represent another feature conferring stress tolerance to C_4 plants. The role of the C_4 pathway in flood tolerance seems to be an important question not only to understand natural distribution patterns of flooding-tolerant species in water-level-fluctuation zones, but also for possible introduction of the C_4 pathway in rice.

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6. Publications of this dissertation

6.1 Photosynthetic acclimation is important for post-submergence recovery of photosynthesis and growth in two riparian species

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Photosynthetic acclimation is important for post-submergence recovery of photosynthesis and growth in two riparian species

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• *Background and Aims* Concomitant increases in O_2 and irradiance upon de-submergence can cause photoinhibition and photo-oxidative damage to the photosynthetic apparatus of plants. As energy and carbohydrate supply from photosynthesis is needed for growth, it was hypothesized that post-submergence growth recovery may require efficient photosynthetic acclimation to increased O_2 and irradiance to minimize photo-oxidative damage. The hypothesis was tested in two flood-tolerant species: a C_3 herb, *Alternanthera philoxeroides*; and a C_4 grass, *Hemarthria altissima*. The impact of low O_2 and low light, typical conditions in turbid floodwater, on post-submergence recovery was assessed by different flooding treatments combined with shading.
• *Methods* Experiments were conducted during 30 d of flooding (waterlogging or submergence) with or without shading and subsequent recovery of 20 d under growth conditions. Changes in dry mass, number of branches/tillers, and length of the longest internodes and main stems were recorded to characterize growth responses. Photosynthetic parameters (photosystem II efficiency and non-photochemical quenching) were determined in mature leaves based on chlorophyll *a* fluorescence measurements.
• *Key Results* In both species growth and photosynthesis recovered after the end of the submergence treatment, with recovery of photosynthesis (starting shortly after de-submergence) preceding recovery of growth (pronounced on days 40–50). The effective quantum yield of photosystem II and non-photochemical quenching were diminished during submergence but rapidly increased upon de-submergence. Similar changes were found in all shaded plants, with or without flooding. Submerged plants did not suffer from photoinhibition throughout the recovery period although their growth recovery was retarded.
• *Conclusions* After sudden de-submergence the C_3 plant *A. philoxeroides* and the C_4 plant *H. altissima* were both able to maintain the functionality of the photosynthetic apparatus through rapid acclimation to changing O_2 and light conditions. The ability for photosynthetic acclimation may be essential for adaptation to wetland habitats in which water levels fluctuate.

Key words: Aerenchyma, *Alternanthera philoxeroides*, flooding, growth, *Hemarthria altissima*, low light, photosynthesis, shade, submergence, waterlogging, wetland plant.

INTRODUCTION

Flooding is detrimental to many terrestrial plants. Soil flooding disrupts the metabolism of mesophytic plants by displacing O_2 from soil pores and promoting O_2 depletion by roots and soil microbes (Drew, 1990). Partial to complete submergence imposes further stress by dramatically reducing gas exchange in above-ground parts of plants, exacerbating O_2 shortage (Vartapetian and Jackson, 1997; Blom, 1999; Colmer and Pedersen, 2008*a*). In addition, low irradiance is another important factor which affects survival of submerged plants. Turbid floodwater considerably lowers light energy reaching submerged plants, causing severe energy limitation (Vervuren *et al.*, 2003; Mommer *et al.*, 2005*a*). For example, at a depth of 25 cm in highly turbid floodwater, light intensity can already be below the photosynthetic light compensation point (Setter *et al.*, 1987). As oxygenic photosynthesis of chloroplasts provides not only carbohydrates but also O_2 in submerged plants (Mommer *et al.*, 2007), low light doubly restricts the underwater performance of plants by decreasing the availability of energy as well as O_2.

Although certain morphological responses are induced by both submergence stress and shade stress, such as increased specific leaf area, vertical leaf orientation or petiole elongation, acclimation and adaptation to submergence are directed predominantly to an improved capacity for gas exchange rather than light capture (Mommer *et al.*, 2005*a*). Altered morphology and anatomy of submerged leaves can enhance underwater gas exchange (Mommer *et al.*, 2005*a*, *b*; Voesenek *et al.*, 2006; Colmer and Pedersen, 2008*b*). Moreover, submerged plants of some species show stimulated elongation of shoot organs to restore contact with the atmosphere above the water surface (Benschop *et al.*, 2005; Jackson, 2008). Formation of longitudinally interconnected gas spaces in roots and shoots (aerenchyma, sometimes also pith cavity), which facilitates internal gas diffusion, is found in many flood-tolerant species (Colmer, 2003; Evans, 2003; Jung *et al.*, 2008).

Along with the abilities to cope with limited O_2, CO_2 and energy availability during flooding, the capacity to quickly resume normal (aerobic) physiological and metabolic activities after the removal of floodwater is another important criterion for flooding tolerance (Gibbs and Greenway, 2003;

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van Eck *et al.*, 2004). Sudden re-exposure to ambient O_2 levels can result in increased production of reactive oxygen species (Wollenweber-Ratzer and Crawford, 1994; Benschop *et al.*, 1998) and acetaldehyde (Zuckermann *et al.*, 1997; Tsuji *et al.*, 2003) in plant tissues acclimated to low O_2 conditions. For submerged plants, this large increase in O_2 concentration can be accompanied by an even larger and abrupt increase in light intensity, which threatens their photosynthetic machinery accustomed to low light conditions. High-light-induced damage to the photosynthetic apparatus (photoinhibition; Osmond, 1994) could impede post-submergence recovery of photosynthesis. Upon returning to terrestrial conditions, plants must overcome these problems of concomitant increase in O_2 and irradiance to grow and store sufficient carbohydrates before the next flooding event.

Although our understanding of the mechanisms of flooding survival in plants has advanced remarkably (Gibbs and Greenway, 2003; Bailey-Serres and Voesenek, 2008), much less is known about post-flooding recovery of growth and photosynthesis in flood-tolerant plants, even though these carbon acquisition and accumulation processes are essential for species performance during flood intervals, and further, for survival of the future flooding. We hypothesized that rapid growth recovery after de-submergence may require the ability to acclimate the photosynthetic apparatus to increased O_2 and irradiance to minimize photo-oxidative damage. This hypothesis was tested in two flood-tolerant perennial species: a C_3 herb, *Alternanthera philoxeroides*; and a C_4 grass, *Hemarthria altissima*. These species were chosen to see whether C_3 and C_4 pathways affect photoacclimatory responses during and after flooding. The impact of low O_2 and low light on growth and photosynthesis recovery was assessed by using three different water levels for flooding (well-drained, soil flooding and complete submergence) with or without shading.

MATERIALS AND METHODS

Plant materials

Alternanthera philoxeroides (Mart.) Griseb. is an exotic invasive perennial C_3 weed in China, which can establish in aquatic, semi-aquatic and terrestrial environments including dry farmland (Allen *et al.*, 2007; Gao *et al.*, 2008). *Hemarthria altissima* (Poir.) Stapf & C.E. Hubb. is a perennial C_4 grass with long spreading stolons and short rhizomes. Plants of *H. altissima* prefer moist soils and occur along river banks, but are also capable of growing in dry land (Wang *et al.*, 2005; Yang *et al.*, 2007). Both species are found in the zone of water-level fluctuations of the Three Gorges Reservoir area of China. For the present study, plants were collected in the Three Gorges Reservoir area and brought to the institute Phytosphäre, Forschungszentrum Jülich, Germany. Plants of *A. philoxeroides* were propagated from cuttings and those of *H. altissima* from tillers. Young plants were then planted in pots (2·2 L, one plant per pot) containing TYP ED 73 soil (mixture of clay and white peat, with pre-mixed fertilizer enough for 2 to 3 months of plant cultivation; Einheitserdewerk, Fröndenberg, Germany) and allowed to grow for 1 month in the glasshouse prior to the experiments.

Plant cultivation and experimental conditions

All experiments were conducted under semi-controlled conditions in the glasshouse. Daily air temperature and relative humidity ranged between 20–22 °C and 40–60 % during the experimental period. Illumination in the glasshouse (SON-T AGRO 400, Philips, Eindhoven, the Netherlands) was automatically turned on when the ambient light intensity outside the glasshouse became <400 μmol photons m^{-2} s^{-1} between 0600 and 2200 h local time. Under such conditions, the photosynthetically active radiation measured at the plant level was typically 200–300 μmol photons m^{-2} s^{-1}. Plants were watered daily during cultivation.

Experimental design

For the experiments, 114 and 130 plants of *A. philoxeroides* and *H. altissima*, respectively, were randomly selected. Three different flooding treatments were applied: 'Control' (no flooding), waterlogging ('Waterlogged', water level at the soil surface) and submergence ('Shaded-submerged', fully submerged under 1-m-deep water and covered with a neutral shade cloth). The shade cloth was used to mimic low-light environments typically found in turbid floodwater; the light intensity beneath the shade cloth was 3–8 μmol photons m^{-2} s^{-1}. The same shading treatment was also applied in combination with Control ('Shaded-control') and Waterlogged treatments ('Shaded-waterlogged') to assess the impact of low irradiance on growth and photosynthesis. Flooding treatments were conducted in plastic tanks (72 and 480 L in volume for waterlogging and submergence, respectively), with six plants per tank. Care was taken to keep plants completely underwater for Shaded-submerged treatment. Following 30 d of different flooding treatments with or without shading, plants were transferred back to the growth conditions and recovery was monitored for 20 d.

Physico-chemical properties of floodwater

Physico-chemical properties of floodwater were checked in the morning (1000–1200 h) at the beginning of the experiment (day 0) and during the submergence treatment (day 20). Water samples were taken immediately before the measurements at the half depth of the floodwater (at approx. 50 cm deep) in three replicate submergence tanks. The inorganic carbon concentrations, including dissolved CO_2, HCO_3^- and CO_3^{2-}, were estimated by using the titration method described by Klee (1998). The level of dissolved O_2 was measured with an O_2 electrode (AM 39, Sensortechnik Meinsberg, Germany). Temperature and pH were also determined to calculate CO_2 and O_2 levels. Mean values are summarized in Table 1.

Light microscopy of transverse sections

Transverse sections of root and stem/culm tissues were hand-sectioned immediately before observation. The positions of the sections were 3 cm above and 3 cm below the soil surface for stem/culm and root, respectively. Sections were observed under a light microscope Zeiss Axiophot2 (Carl

TABLE 1. *Physico-chemical properties of floodwater in submergence tanks*

Parameter	Day 0	Day 20
Temperature (°C)	19·9	18·2
pH	6·7	6·5
CO_2 (μmol L^{-1})	136 ± 2	186 ± 36
HCO_3^- (μmol L^{-1})	331 ± 5	254 ± 8
O_2 (μmol L^{-1})	228 ± 1	219 ± 6

All values are means (± s.e.) of three individual measurements from three flooding tanks. The amounts of CO_3^{2-} detected in the floodwater were below 1 μmol L^{-1}.

Zeiss, Jena, Germany) and images were taken by a digital camera (D200, Nikon, Tokyo, Japan).

Growth analyses

Dry weight data were collected at the beginning and at the end of the flooding treatments (day 0 and 30, respectively) as well as after 10 and 20 d of recovery under the growth conditions (day 40 and 50, respectively). At each sampling point, 8–10 plants were harvested for every treatment. The plant materials were put in an oven at 75 °C until a constant mass was reached. The parameters used for growth analyses were: above- and below-ground dry weight, total number of branches/tillers, and length of the longest internodes and main stem.

Chlorophyll a *fluorescence measurements*

Chlorophyll (Chl) *a* fluorescence analysis can provide information about photoinhibition, photochemical efficiency and photoprotective energy dissipation of photosystem II (PSII) (Schreiber and Bilger, 1993; Maxwell and Johnson, 2000). Measurements were performed in the glasshouse in the morning (0900–1100 h) every other day during the experiments. For Shaded-submerged plants, no measurements were taken during submergence. Fluorescence was measured on youngest, fully expanded leaves (i.e. mature leaves closest to the growth zone of the shoot) by using a Handy PEA instrument (Hansatech Instruments, King's Lynn, UK). The maximal and minimal fluorescence intensity of dark-adapted leaves (F_m and F_0, respectively) were determined after 30 min of dark adaptation by using leaf clips. The maximal and steady-state fluorescence intensity of light-adapted leaves ($F_{m'}$ and F_s, respectively) were determined after 4·5 min illumination at 800 μmol photons m^{-2} s^{-1} with the built-in red light source of the PEA instrument. The intensity and duration of the saturation pulse applied to determine F_m and $F_{m'}$ were 3500 μmol photons m^{-2} s^{-1} and 1 s, respectively. The maximal quantum yield of PSII in the dark (F_v/F_m) was calculated as $F_v/F_m = (F_m - F_0)/F_m$, the effective quantum yield of PSII in the light ($\Delta F/ F_{m'}$) as $\Delta F/ F_{m'} = (F_{m'} - F_s)/F_{m'}$, and the non-photochemical energy quenching in PSII (NPQ) as $NPQ = (F_m - F_{m'})/F_{m'}$ (Maxwell and Johnson, 2000).

Statistical data analysis

Growth data were checked for normal distribution and equal variance between populations by using SigmaStat 2·0 (SPSS Inc., Chicago, IL, USA). The data were then statistically tested for differences between treatments (having the same sampling size) by analysis of variance (one-way ANOVA) or for differences between sampling times (having slightly different sampling sizes) by the Kruskal–Wallis test (both in SigmaStat 2·0). As the difference in sampling size was minimal in our experiment ($n = 8–10$), the Kruskal–Wallis test gave essentially the same results as one-way ANOVA.

RESULTS

Changes in physico-chemical properties of floodwater during the submergence treatment

Shaded-submerged treatment decreased the pH value of floodwater (Table 1). Accordingly, the HCO_3^- concentration in the floodwater decreased slightly; by contrast, the CO_2 concentration increased. When CO_2 and HCO_3^- levels were added together, there was a minor decrease in the total inorganic carbon from day 0 to day 20 (from 467 to 440 μmol L^{-1}). Amounts of CO_3^{2-} detected in the floodwater were negligible (<1 μmol L^{-1}, data not shown). The dissolved O_2 concentration changed little in the submergence tanks.

Shoot and root anatomy

Both *A. philoxeroides* and *H. altissima* are terrestrial plants which can grow well in wetland. Transverse sections prepared from shoot/culm and root tissues of Shaded-submerged plants showed a well-developed pith cavity and aerenchyma (Fig. 1), suggesting structural adaptation to wetland habitats. The samples were taken at 30 mm above and 30 mm below the soil surface for stems/culms and roots, respectively. These tissues were formed during cultivation in the glasshouse, i.e. they are not aquatic shoots/culms or roots which grew during Shaded-submerged treatment. In fact, different flooding treatments did not visibly alter the number and/or size of pith cavity and aerenchyma during the 30-d flooding treatments, and similar anatomy was also observed in well-drained Control plants (data not shown). Thus, it seems that these plants have porous shoots and roots constitutively.

Growth responses to flooding with or without shading

Growth responses to different flooding treatments with or without shading were compared in *A. philoxeroides* and *H. altissima* during 50 d of the flooding and recovery experiments. Above- and below-ground dry mass increased in Control plants of *A. philoxeroides* >20-fold during the experiment (Fig. 2A and B, respectively). Waterlogged treatment resulted in a significantly smaller dry mass increase compared with Control. Plants of *A. philoxeroides* have a repent growth form, and the contact of shoots with the floodwater in the waterlogging tank stimulated the formation of adventitious roots at stem nodes. On day 30 these adventitious roots amounted to 7–10 % of the total root fresh weight for Waterlogged plants (data not shown). When shaded (Shaded-control, Shaded-waterlogged and Shaded-submerged), *A. philoxeroides* virtually did not accumulate dry mass, with or without flooding. Yet the dry mass increase started

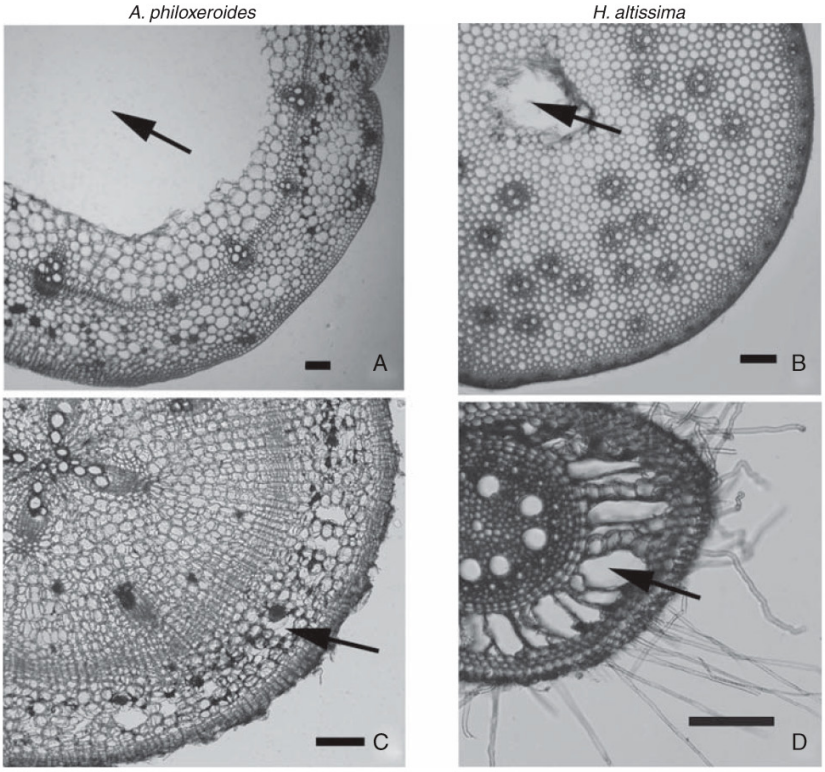

FIG. 1 Transverse sections of stem/culm (A, B) and root tissues (C, D) of Shaded-submerged plants of *A. philoxeroides* (A, C) and *H. altissima* (B, D). Sections were made at 30 mm above and 30 mm below the soil surface for stems/culms and roots, respectively. Note that these tissues were formed during the cultivation before the flooding treatment, not during the flooding treatment. Arrows indicate pith cavity and aerenchyma. Scale bars = 0·2 mm.

between day 30 and 50, with the recovery in Shaded-submerged plants being slower than in other shaded plants both above and below ground. The root-to-shoot ratio increased in Control plants of *A. philoxeroides* with increasing plant size (Fig. 2C), presumably due to 'ontogenic drift' (Geng *et al.*, 2007). The ratios strongly decreased in Shaded-control and Shaded-waterlogged plants from day 0 to 30, whereas they did not change significantly in Waterlogged and Shaded-submerged plants. The ratio then increased during the recovery period in all treatments (except for Control) to reach Control-like values by day 50 in all but Shaded-submerged plants.

The Control plants of *H. altissima* accumulated much less dry mass than Control plants of *A. philoxeroides*: <10-fold for above-ground and <6-fold for below-ground (Fig. 2D and E, respectively) dry mass. As in *A. philoxeroides*, dry mass accumulation of Waterlogged plants was less than in Control plants, and shading allowed no increment of above- and below-ground dry mass, with or without flooding. Accumulation of dry mass started in shaded plants after day 30, but the major increase occurred between day 40 and 50. The apparent lack of change in above-ground dry mass of Shaded-submerged plants between day 30 and 40 (Fig. 2D) actually contained growth of some new leaves which replaced mature leaves lost shortly after de-submergence. Unlike in *A. philoxeroides*, the root-to-shoot ratio decreased in Control plants of *H. altissima* from day 0 to 30 (Fig. 2F). While retarding the dry mass increase, Waterlogged treatment did not affect the root-to-shoot balance in *H. altissima*. Shading slowed the decrease in root-to-shoot ratio, but the values for Shaded-control and Shaded-waterlogged plants approached those of Control and Waterlogged plants within the first 10 d of recovery. Only Shaded-submerged plants maintained higher values throughout the experiment. In general, the root-to-shoot ratio of *H. altissima* was about half that of *A. philoxeroides* on day 50 (Fig. 2C, F), indicating that relative dry mass allocation to shoots was greater in *H. altissima* than in *A. philoxeroides*, and vice versa for relative allocation to roots, during the experiments.

The responses of above-ground growth patterns to different treatments were further characterized by comparing the number of branches (*A. philoxeroides*) or tillers (*H. altissima*). The total number of branches increased from day 0 to 30 in Control and Waterlogged plants of *A. philoxeroides* by about four- and three-fold, respectively (Fig. 3A). Shading suppressed the formation of new branches, but Shaded-control and Shaded-waterlogged plants were able to produce many new branches after the end of the treatments.

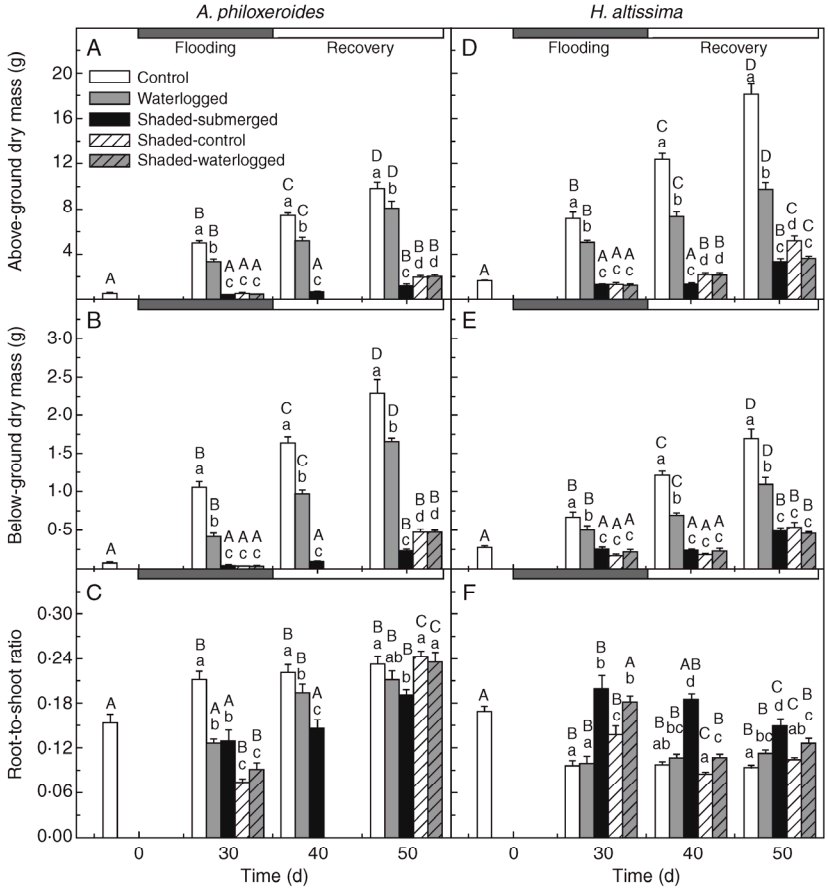

FIG. 2 Changes in above-ground dry mass (A, D), below-ground dry mass (B, E) and root-to-shoot ratio (C, F) of *A. philoxeroides* (A–C) and *H. altissima* (D–F) following the different flooding treatments with or without shading. Each value is a mean of 8–10 plants (±s.e.). All plants were under the Control condition on day 0. For *A. philoxeroides*, plants of Shaded-control and Shaded-waterlogged were not harvested on day 40. Different lower-case letters indicate statistically significant differences ($P < 0{\cdot}05$ by one-way ANOVA) between the treatments at each time point. Different upper-case letters indicate statistically significant differences ($P < 0{\cdot}05$ by the Kruskal–Wallis test) between the measurement time points for each treatment.

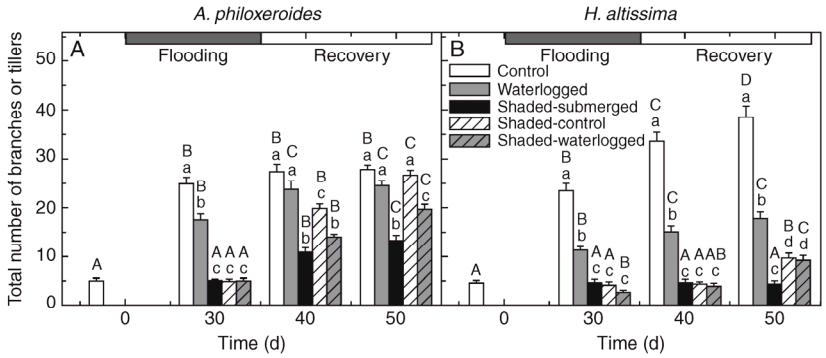

FIG. 3 Changes in the total branch/tiller number of *A. philoxeroides* (A) and *H. altissima* (B) following the different flooding treatments with or without shading. Each value is a mean of 8–10 plants (±s.e.). All plants were under the Control condition on day 0. Different lower-case letters indicate statistically significant differences ($P < 0{\cdot}05$ by one-way ANOVA) between the treatments at each time point. Different upper-case letters indicate statistically significant differences ($P < 0{\cdot}05$ by the Kruskal–Wallis test) between the measurement time points for each treatment.

These plants had as many branches as Control and Waterlogged plants by day 50 even though their above-ground dry mass was still much less (Fig. 2A). Recovery of branching was far slower in Shaded-submerged plants, having only half as many branches as Control plants at the end of the recovery period (Fig. 3A). The above-ground growth of *H. altissima* was characterized by a large increase (about eight-fold in Control plants) in total tiller number (Fig. 3B). Tiller formation in this grass species responded very sensitively to flooding as well as to shading treatments; it was strongly impaired in Waterlogged treatment and was completely halted until day 40 in Shaded-control and Shaded-waterlogged treatments. Shaded-submerged plants did not grow new tillers during 20 d of the recovery period.

Although suppressing the accumulation of dry mass and formation of new branches, 30 d of submergence strikingly enhanced elongation of internodes in *A. philoxeroides* (Fig. 4A). Even though dry mass accumulation was similarly inhibited in all shaded plants, only Shaded-submerged plants had longer internodes than Control plants (by approx. 20 %) on day 30. By elongating the existing internodes (i.e. without formation of new internodes) Shaded-submerged plants increased the total length of the main stem by about 40 cm (or 200 % of the initial length on day 0), while the corresponding increment in Shaded-control and Shaded-waterlogged plants was only about 15 cm (Fig. 4B). The main stems of Control and Waterlogged plants grew much longer than those of shaded plants, but this was achieved by making new internodes (data not shown). In marked contrast, variations in the internode length among different treatments were minimal for *H. altissima* (Fig. 4C). The main culms grew longer only in Control and Waterlogged plants, which produced several new internodes during the period (Fig. 4D).

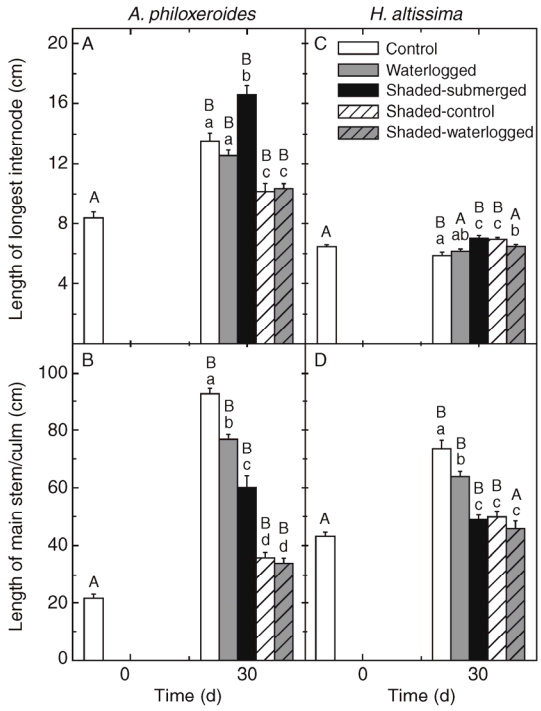

FIG. 4. Length of the longest internodes (A, C) and the main stem/culm (B, D) for *A. philoxeroides* (A, B) and *H. altissima* (C, D) at the end of the different flooding treatments with or without shading. Each value is a mean of 8–10 plants (± s.e.). All plants were under the Control condition on day 0. Different lower-case letters indicate statistically significant differences ($P < 0{\cdot}05$ by one-way ANOVA) between the treatments at each time point. Different upper-case letters indicate statistically significant differences ($P < 0{\cdot}05$ by the Kruskal–Wallis test) between the measurement time points for each treatment.

Photosynthetic responses to flooding with or without shading

The above results demonstrated the ability of *A. philoxeroides* and *H. altissima* to resume growth and dry mass accumulation relatively quickly after de-submergence. According to the present hypothesis, the photosynthetic apparatus of these plants must be able to cope with extreme changes in O_2 and light. However, growth recovery was retarded in Shaded-submerged plants compared with Shaded-control and Shaded-waterlogged plants (Figs 2 and 3), which may reflect slower acclimation and recovery of photosynthesis due to detrimental effects of submergence on the photosynthetic apparatus.

The maximal quantum yield of PSII (F_v/F_m) indicated no significant photoinhibition in Waterlogged plants of *A. philoxeroides* throughout the experiment (Fig. 5A). Shaded-submerged plants had slightly lower F_v/F_m values shortly after de-submergence, but F_v/F_m fully recovered in these plants on day 39. Shading quickly increased F_v/F_m to the maximal level (approx. 0·84) in both Shaded-control and Shaded-waterlogged plants, whereas the transfer back to the growth conditions transiently decreased F_v/F_m to the values found in Shaded-submerged plants (Fig. 5B). Thereafter, F_v/F_m fully recovered in Shaded-control and Shaded-waterlogged plants by day 37, i.e. 2 d earlier than in Shaded-submerged plants. In contrast to the situation in *A. philoxeroides*, Waterlogged treatment markedly decreased F_v/F_m in *H. altissima* in the first 20 d, suggesting some photoinhibitory damage to PSII (Fig. 5C). Yet F_v/F_m started to recover in the last 10 d of Waterlogged treatment to become comparable with that of Control plants 3 d after draining the soil. F_v/F_m values of Shaded-submerged plants during the recovery period were as high as those of Control plants. The shading responses of F_v/F_m in *H. altissima* were generally the same as described for *A. philoxeroides*, albeit with less pronounced changes (Fig. 5D). Full recovery of F_v/F_m was found on day 37 in Shaded-control and on day 39 in Shaded-waterlogged treatment.

Whereas F_v/F_m indicated little PSII photoinhibition in all but Waterlogged plants of *H. altissima*, the effective quantum yield of PSII ($\Delta F/F_{m'}$) revealed a strikingly reduced capacity of shaded plants to utilize light energy under illumination (Fig. 6). Waterlogged treatment had no significant effect on $\Delta F/F_{m'}$ in *A. philoxeroides* (Fig. 6A). The shading treatment caused a rapid and dramatic decrease in $\Delta F/F_{m'}$ in Shaded-control and Shaded-waterlogged plants to a level as low as that measured in Shaded-submerged plants (Fig. 6B). Recovery of $\Delta F/F_{m'}$ started in all shaded plants of *A. philoxeroides* within 3 d after the end of the treatments,

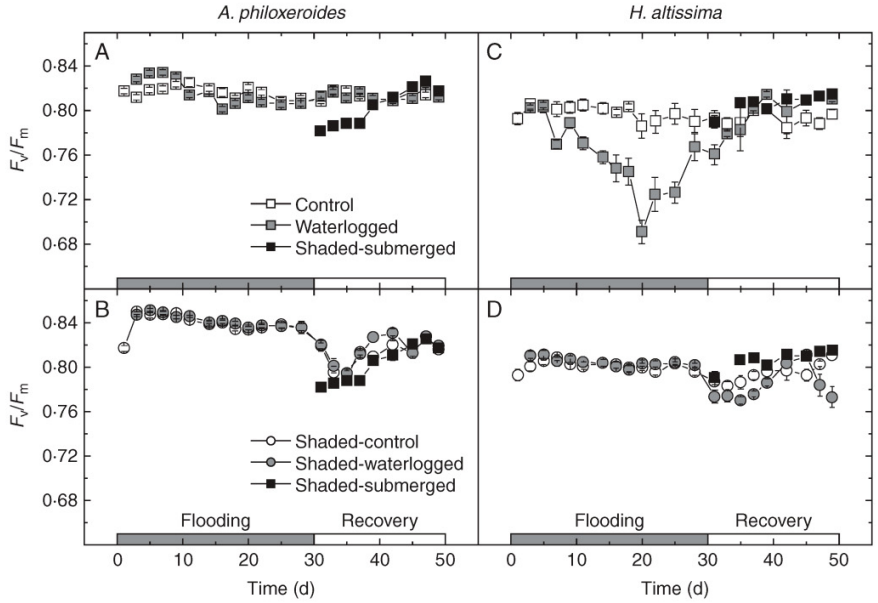

FIG. 5. Changes in the maximal quantum yield of photosystem II (F_v/F_m) in dark-adapted leaves of *A. philoxeroides* (A, B) and *H. altissima* (C, D) during and after different flooding treatments with or without shading. Data from Shaded-submerged plants are shown in comparison with Control and Waterlogged plants (A, C) or with Shaded-control and Shaded-waterlogged plants (B, D). Measurements for Shaded-submerged plants were started at the end of the submergence treatment on day 30. Symbols are mean values (± s.e., $n = 4–5$).

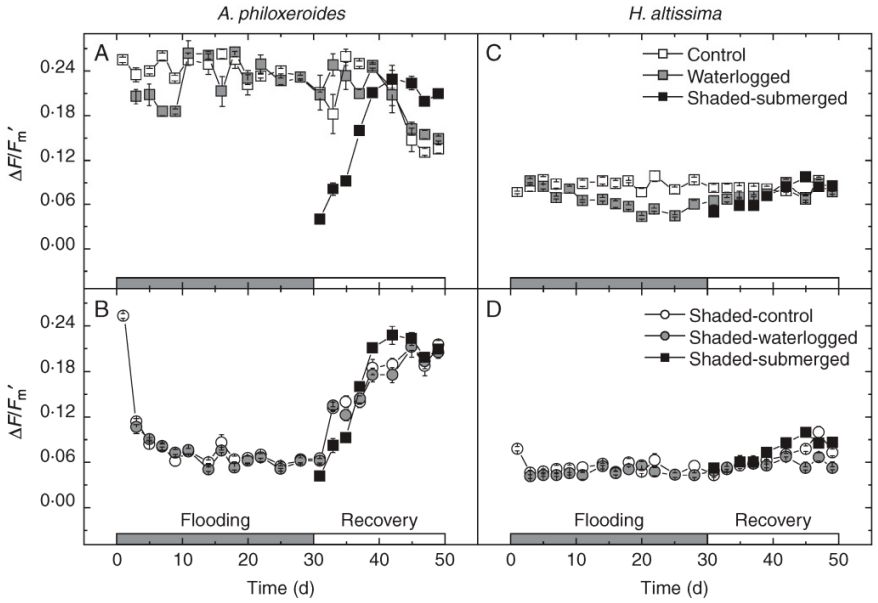

FIG. 6. Changes in the effective quantum yield of photosystem II ($\Delta F/F_m'$) in leaves of *A. philoxeroides* (A, B) and *H. altissima* (C, D) during and after different flooding treatments with or without shading. Values were obtained after 4·5 min of illumination at 800 μmol photons m^{-2} s^{-1}. Data from Shaded-submerged plants are shown in comparison with Control and Waterlogged plants (A, C) or with Shaded-control and Shaded-waterlogged plants (B, D). Measurements for Shaded-submerged plants were started at the end of the submergence treatment on day 30. Symbols are mean values (± s.e., $n = 4–5$).

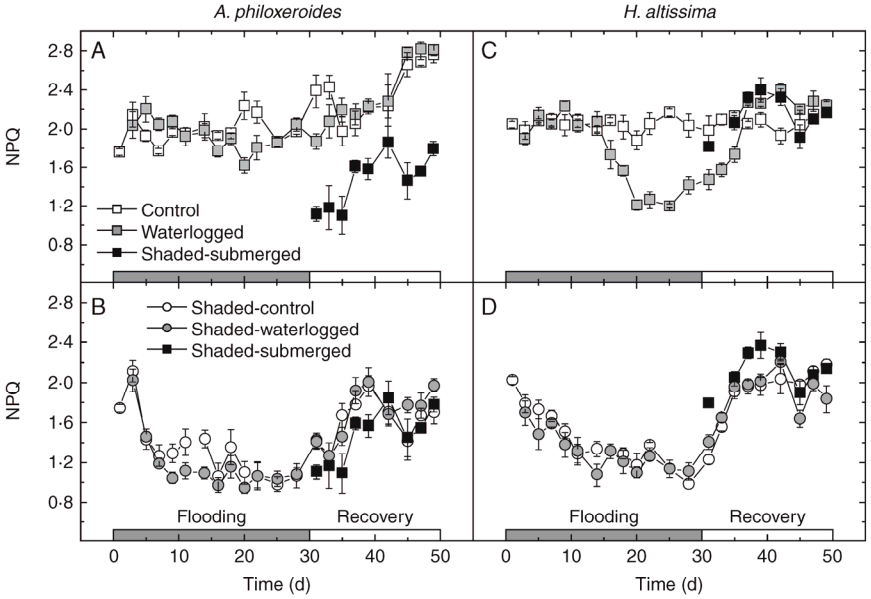

FIG. 7. Changes in non-photochemical energy quenching (NPQ) in leaves of *A. philoxeroides* (A, B) and *H. altissima* (C, D) during and after different flooding treatments with or without shading. Values were obtained after 4·5 min of illumination at 800 μmol photons m^{-2} s^{-1}. Data from Shaded-submerged plants are shown in comparison with Control and Waterlogged plants (A, C) or with Shaded-control and Shaded-waterlogged plants (B, D). Measurements for Shaded-submerged plants were started at the end of the submergence treatment on day 30. Symbols are mean values (± s.e., $n = 4–5$).

although it took approx. 10 d to recover fully. In comparison, *H. altissima* generally had much lower photochemical efficiency than *A. philoxeroides* (Fig. 6C, D). The pronounced negative effect of Waterlogged treatment was also visible in $\Delta F/F_{m'}$ of this species (Fig. 6C). Unlike F_v/F_m, $\Delta F/F_{m'}$ of *H. altissima* was substantially reduced to approx. 60 % of Control plants at the beginning of Shaded-control and Shaded-waterlogged treatments, or at the end of Shaded-submerged treatment (Fig. 6D). For both species, recovery of $\Delta F/F_{m'}$ in Shaded-submerged treatment was not slower than in Shaded-control and Shaded-waterlogged treatments.

When a large fraction of absorbed light energy becomes excessive due to low $\Delta F/F_{m'}$, leaves may up-regulate NPQ to protect the photosynthetic apparatus and minimize photoinhibition. Shading substantially diminished the NPQ capacity in both *A. philoxeroides* and *H. altissima*, but rapid recovery was observed after day 30 (Fig. 7). The changes in NPQ paralleled the large variations in F_v/F_m and $\Delta F/F_{m'}$ in Waterlogged plants of *H. altissima* (Fig. 5C). Again, the NPQ recovery of Shaded-submerged plants did not differ from that in other shaded plants for both species (Fig. 7B, D).

DISCUSSION

Post-submergence recovery of growth and photosynthesis

During the 20-d recovery period the total dry mass of Shaded-submerged plants increased by 255 and 148 % in *A. philoxeroides* and *H. altissima*, respectively. This shows that *A. philoxeroides* could achieve a greater relative growth rate than *H. altissima* after de-submergence. Similar increases in dry mass, ranging between 22·1 and 305·6 %, have been reported for some species found in low-elevation grassland along the Rhine River during 3 weeks of recovery after 30 d of submergence (van Eck *et al.*, 2004).

Post-submergence growth recovery, as observed in dry mass (Fig. 2) and formation of new branches and tillers (Fig. 3), was preceded by recovery of $\Delta F/F_{m'}$ and NPQ in both *A. philoxeroides* and *H. altissima* (Figs 5–7). Yet the slowest growth resumption of Shaded-submerged plants was not accompanied by a corresponding delay in photosynthetic recovery; the fluorescence parameters indicated comparable recovery in all shaded plants after day 30 (Figs 5–7). This means that the retarded growth recovery of Shaded-submerged plants was not a result of slow photosynthetic acclimation. The leaves of these wetland plants were able to quickly adjust the capacities of photosynthetic electron transport and dissipation to prevailing light environments (Figs 6 and 7), and there was no sign of severe photoinhibition after the end of the submergence and/or shading treatments (Fig. 5). The ability for flexible photosynthetic acclimation may be essential for wetland plants living in fluctuating water, in which the availability of light, CO_2 and O_2 changes dramatically and periodically.

There was no significant difference in post-submergence recovery patterns of growth and photosynthesis between the C_3 plant *A. philoxeroides* and the C_4 plant *H. altissima* in the present experiments. Thus, both the C_3 and the C_4 pathway seem to be compatible with the metabolic and morphological adjustment involved in flooding tolerance. The

only differences found between the two species were the generally lower $\Delta F/F_{m'}$ in *H. altissima* and its higher sensitivity to waterlogging. It is likely that the lower PSII efficiency in the light (Fig. 6C, D) contributed to the smaller relative dry mass increase in *H. altissima* compared with *A. philoxeroides* during recovery. Decreased stomatal conductance under soil flooding, as has been demonstrated in some woody species (Mielke *et al.*, 2003), may have restricted leaf gas exchange in Waterlogged plants of *H. altissima*, resulting in photoinhibition under the ambient light in the glasshouse (Fig. 5C).

What, then, could be the reason for the slower growth recovery in Shaded-submerged plants? Shaded-submerged plants may have consumed more carbohydrates and energy than Shaded-control and Shaded-waterlogged plants during the 30-d treatments, leading to severely reduced carbohydrate availability for growth at the beginning of the recovery period. Additionally, or alternatively, limited O_2 supply may have deteriorated root functionality (Vartapetian and Jackson, 1997) to delay growth recovery and cause loss of leaves upon de-submergence. Post-submergence injuries due to accumulation of reactive oxygen species (Wollenweber-Ratzer and Crawford, 1994; Benschop *et al.*, 1998) and acetaldehyde (Zuckermann *et al.*, 1997; Tsuji *et al.*, 2003) are also potential factors contributing to growth retardation. These possible causes of post-submergence growth retardation in *A. philoxeroides* and *H. altissima* will be investigated in future studies.

Flooding tolerance in A. philoxeroides *and* H. altissima

Flood-tolerant species can grow at low elevations along the floodplain while less flood-tolerant species are confined to higher elevations of the floodplain gradient (van Eck *et al.*, 2004). The two riparian species examined here, *A. philoxeroides* and *H. altissima*, occur in low-elevation sites of river banks and are capable of enduring long-term submergence (Wang *et al.*, 2008*a*, *b*). However, different survival rates, 50 and 90 % for *A. philoxeroides* and *H. altissima*, respectively, have been reported after 180 d of complete submergence (Wang *et al.*, 2008*a*, *b*). Although the recovery of dry mass accumulation after 30 d of submergence was greater in *A. philoxeroides* than in *H. altissima* in the present study, the better success of *H. altissima* than *A. philoxeroides* under very long submergence, as in the previous studies by Wang *et al.* (2008*a*, *b*), may be explained by the contrasting growth responses of these two plants during submergence.

Submergence enhanced stem elongation in *A. philoxeroides* (Fig. 4A, B), a morphological response observed in certain flood-tolerant species (Mommer *et al.*, 2005*b*; Jackson, 2008). Rapid stem elongation serves to regain its contact with the atmosphere or well-oxygenated upper water layers. Once contact with the atmosphere is established in relatively shallow water, these plants can perform efficient photosynthesis and survive prolonged submergence (Voesenek *et al.*, 2004). Strongly elongated stems and petioles are also found in plants under shade environments ('shade avoidance'; Ballaré *et al.*, 1999; Valladares, 2003; Franklin and Whitelam, 2005). However, plants of *A. phioloxeroides* in Shaded-control and Shaded-waterlogged treatments did not exhibit strong stem elongation in the present study (Fig. 4A, B). This shows that the elongation response of *A. phioloxeroides* was induced by submergence, not by shading, probably via ethylene signalling (Benschop *et al.*, 2005; Mommer *et al.*, 2005*a*; Cox *et al.*, 2006; Jackson, 2008). It has been demonstrated that submergence-induced elongation is different from shade-induced elongation; the former can occur at relatively high light intensities (e.g. 100 μmol photons m^{-2} s^{-1}) and high red/far-red ratios (Pierik *et al.*, 2005) whereas the latter is induced by low red/far-red ratios (Ballaré *et al.*, 1999; Valladares, 2003; Franklin and Whitelam, 2005).

The escape strategy requires not only contact with the atmosphere but also porous shoots and roots to allow internal gas diffusion (Fig. 1A, C; Colmer, 2003; Evans, 2003; Jung *et al.*, 2008). The importance of petiole porosity for the escape strategy has been recently demonstrated in species of *Rumex* (Pierik *et al.*, 2009); whereas *R. palustris*, having sufficient aerenchyma in the petiole, accumulated biomass in shoots during submergence, *R. acetosa*, a congeneric species without such anatomy, did not. In shallow floodwater, in which elongated shoots of *A. philoxeroides* can reach the atmosphere, this species may outperform *H. altissima* by the 'escape' strategy, even though the submergence-induced stem elongation may be costly under energy limitation. With increasing depth of floodwater and decreasing availability of light, the advantage of the escape strategy may be outweighed by its cost.

Plants of *H. altissima* did not elongate internodes in response to submergence (Fig. 4C). Instead of trying to escape, this plant may have adopted a 'quiescence' strategy to survive long submergence (Geigenberger, 2003; Voesenek *et al.*, 2006). There were indications that *H. altissima* is not only flood-tolerant but also shade-tolerant, a beneficial trait for a quiescence strategy (Geigenberger, 2003; Voesenek *et al.*, 2006). For example, the capacity of PSII electron transport measured in *H. altissma* was less than half that of *A. philoxeroides* (Fig. 6). Further studies are needed to elucidate the physiological mechanisms of the pronounced long-term flood tolerance of *H. altissima*.

CONCLUDING REMARKS

The C_3 plant *A. philoxeroides* and the C_4 plant *H. altissima* are both able to maintain the functionality of the photosynthetic apparatus after de-submergence through rapid acclimation to changing O_2 and/or light conditions. The ability for photosynthetic acclimation may be essential for adaptation to wetland habitats in which the water level fluctuates. Although limited light availability had the major impact on photosynthesis and growth in our experiments, submergence delayed growth recovery more strongly than shading or the combination of soil flooding and shading. Physiological and metabolic factors determining the extent of post-submergence growth retardation in flood-tolerant species deserve further investigation.

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6.2 Recovery dynamics of growth, photosynthesis and carbohydrate accumulation after de-submergence: A comparison between two wetland plants showing escape and quiescence strategies

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Own contribution

- Experiments
- Data analysis (with co-authors)
- Preparation of manuscript (with co-authors)

Recovery dynamics of growth, photosynthesis and carbohydrate accumulation after de-submergence: A comparison between two wetland plants showing escape and quiescence strategies

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ABSTRACT

Background and Aims The capacity for fast growth recovery after de-submergence is important for establishment of riparian species in water-level-fluctuation zone. We investigated recovery patterns of two wetland plants, *Alternanthera philoxeroides* and *Hemarthria altissima*, showing 'escape' and 'quiescence' responses, respectively, during submergence.

Methods Leaf and root growth and photosynthesis were monitored continuously during 10 d of recovery following 20 d of complete submergence. Above- and belowground dry weight as well as carbohydrate concentrations were measured several times during the experiment.

Key Results Both species remobilized stored carbohydrate during submergence. Although enhanced internode elongation depleted the carbohydrate storage in *A. philoxeroides* during submergence, this species resumed leaf growth 3 d after de-submergence concomitant with restoration of the maximal photosynthetic capacity. In contrast, some sucrose was conserved in shoots of *H. altissima* during submergence, which promoted rapid re-growth of leaves 2 d after de-submergence and earlier than the full recovery of photosynthesis. The recovery of root growth was delayed by 1 – 2 d compared with leaves in both species.

Conclusions Submergence tolerance of the escape and quiescence strategies entails not only the corresponding regulation of growth, carbohydrate catabolism and energy metabolism during submergence but also coordinated recovery of photosynthesis, growth and carbohydrate partitioning following de-submergence.

Key words: *Alternanthera philoxeroides*, carbohydrate, flooding, *Hemarthria altissima*, leaf growth, root growth, shade, submergence, wetland plant.

INTRODUCTION

Submergence stress is one of the common environmental challenges for plants in natural and artificial ecosystems. It is detrimental for many terrestrial plants, causing ultimate death (Voesenek *et al*., 2006). The negative impact of submergence on terrestrial plants is mainly related to slow gas diffusion and low light intensity, which severely restricts the energy and carbohydrate availability (Bailey-Serres and Voesenek, 2008; Colmer and Voesenek, 2009). Especially, poor light transmission in highly turbid floodwater or under thick algal growth can aggravate the energy and carbohydrate deficiency in submerged plants (Whitton *et al*., 1988) not only by depriving light but also by inhibiting photosynthesis through structural and functional impairment of chloroplasts (Panda *et al*., 2006).

Submergence-tolerant species are able to alleviate some of these adverse effects by changing the morphology and anatomy of shoots and roots, which facilitates uptake and internal transport of CO_2 and O_2 (Armstrong, 1979; Mommer *et al*., 2007; Colmer and Pedersen, 2008), and by switching from aerobic to anaerobic respiration for energy conversion, depending on the availability of O_2 (Gibbs and Greenway, 2003). Nevertheless, roots of submerged plants often suffer from energy and carbohydrate shortage (Gaynard and Armstrong, 1987; Farrar and Jones, 2000) along with damage caused by reduced components (Mn^{2+}, Fe^{2+} and S^{2-}) and volatile organic acids (propionic and butyric acids) accumulating in flooded soil (Jackson and Drew 1984; Armstrong and Armstrong, 1999; Greenway *et al*., 2006). Tissue injuries which developed underwater can be intensified as the water recedes and shoots become re-exposed to the atmosphere (Sarkar *et al*., 2006). A sudden increase in O_2 upon de-submergence exacerbates the conditions unless plants can cope with excessive formation of reactive oxygen species and toxic oxidative products in different cells and organs (Sarkar *et al*., 2001; Blokhina *et al*., 2003; Santosa *et al*., 2007). The direct sunlight also threatens leaves accustomed to low-light underwater environments, causing photoinhibition to the photosynthetic apparatus (Osmond, 1994).

Submergence tolerance of terrestrial plants can be attributed to two contrasting strategies: the 'escape' and 'quiescence' strategies (Perata and Voesenek, 2007; Bailey-Serres and Voesenek, 2008). The plants with the escape strategy respond to submergence by enhanced shoot elongation to regain contact with the atmosphere, whereas those with the quiescence strategy conserve energy and carbohydrate by restraining growth. The costly escape strategy is

advantageous in shallow floodwater, in which the shoot elongation allows plants to re-establish air contact; failure to do so results in accelerated depletion of carbohydrate reserves. The quiescence strategy, on the other hand, can save energy and resource during submergence, which could positively affect the survival rate and generation of new tissues after de-submergence (Das *et al*., 2005; Fukao *et al*., 2006; Panda *et al*., 2008), provided that the flooding period is not too long.

The capacity for fast growth recovery after de-submergence, withstanding the weakness and injuries from submergence and the difficulties in re-acclimating to terrestrial conditions, is crucial for successful establishment of riparian species in water-level-fluctuation zones. While the regulatory mechanisms (Fukao *et al.*, 2006; Xu *et al.*, 2006; Hattori *et al.*, 2009) and physiological effects of the two flood-tolerance strategies (e.g. Groeneveld and Voesenek, 2003; Das *et al*., 2005; Dixon *et al*., 2006) have been investigated in different plants during submergence, relatively few studies have examined in detail the recovery processes and responses that happen after de-submergence. How quickly can flood-tolerant plants resume biomass accumulation and growth after return to terrestrial conditions, and how do they allocate the biomass between above- and belowground parts during growth recovery? Do the recovery patterns of these plants after de-submergence resemble the recovery from shade (very low light)? To address these questions, we studied the recovery behaviours of two wetland species, *Alternanthera philoxeroides* and *Hemarthria altissima*, which exhibit typical responses of the escape and quiescence strategies, respectively, during submergence (see Fig. 4 in Luo *et al*., 2009). In an attempt to infer interactions between recovery of photosynthesis, growth and carbohydrate partitioning, these processes were continuously monitored in shoots and roots of the two species for 10 d following 20 d of complete submergence under very low light intensities, mimicking the typical conditions in deep or turbid floodwater. Additionally, some of the plants were subjected to low-light stress, instead of submergence stress, to distinguish the responses and effects induced by submergence from those by very low light.

MATERIALS AND METHODS

Plant materials and growth condition

Alternanthera philoxeroides (Mart.) Griseb. is a perennial C_3 weed which can establish in aquatic, semi-aquatic and terrestrial environments; in terrestrial environments it has a creeping growth habit. *Hemarthria altissima* (Poir.) Stapf & C.E. Hubb. is a perennial stoloniferous C_4 grass which prefers moist soil but is capable of growing in dry land. Both species are distributed in the water-level-fluctuation zone of the Three Gorges Reservoir area in China. For the present study, plants were collected in the Three Gorges Reservoir area and brought to the institute Phytosphäre, Forschungszentrum Jülich, Germany. Plants of *A. philoxeroides* were propagated from cuttings and those of *H. altissima* from tillers. Young plants of *A. philoxeroides* and *H. altissima* were planted in pots (3.8 L, 15 cm diameter × 22 cm, one plant per pot) or rhizotrons (3.6 L, 30 × 2 × 60 cm, one plant per rhizotron) filled with black peat soil (Graberde; Plantaflor Humus, Vechta, Germany) containing the following nutrients according to the specification by the company: N 120 mg L^{-1}, P_2O_5 120 mg L^{-1}, K_2O 170 mg L^{-1}.

Plants were grown for a month under semi-controlled conditions in the glasshouse and watered daily. The air temperature and relative humidity in the glasshouse ranged between 20 – 22°C and 40 – 60%, respectively, during the cultivation and experimental period. Illumination in the glasshouse (SON-T AGRO 400, Philips) was automatically turned on when the ambient light intensity outside the glasshouse became < 400 μmol photons m^{-2} s^{-1} (photosynthetically active radiation, PAR) between 0600 and 2200 h local time. Under such conditions, PAR measured at the plant level was 200 – 300 μmol photons m^{-2} s^{-1} for potted plants (placed on a table at 1 m from the floor) and 150 – 250 μmol photons m^{-2} s^{-1} for plants growing in rhizotrons (placed on the floor). After a month of cultivation, experiments were started in the glasshouse.

Experimental design

For each species a total of 105 plants (96 plants in pots and nine plants in rhizotrons) were randomly selected for the experiments. Experiment with potted plants: two species × three treatments × four replicate plants at each measurement time; chlorophyll *a* fluorescence was

continuously monitored during the 10-d recovery and samples for dry weight and carbohydrate analysis were harvested at three or six time points, respectively, during the experiment. Experiment with plants in rhizotrons: two species × three treatments × three replicate plants at each measurement time; leaf and root growth were continuously measured during the recovery. At the beginning of the experiments (on day 0), plants were divided into three groups and subjected to the following treatments: (1) plants kept under the growth condition in the glasshouse throughout the experiments ('control'), (2) plants placed under a neutral shade cloth (without changing the spectral quality of light) in the glasshouse between day 0 and 20 and transferred back to the control condition on day 20 ('de-shaded'), and (3) plants fully submerged under 1-m deep water (tap water) in 480-L plastic tanks and covered with a neutral shade cloth in the glasshouse between day 0 and 20 and transferred back to the control condition on day 20 ('de-submerged'). No mineral was added to the water during the submergence treatment. The PAR reaching the plants under the shade clothes was < 10 μmol photons m^{-2} s^{-1}, mimicking very low light intensities under deep or turbid floodwater. In the submergence tanks, shade clothes were spanned at about 1 cm below the water surface to keep the submerged plants completely underwater throughout the treatment. These treatments of very low light and complete submergence were applied to simulate the conditions in deep or turbid floodwater typically found in the water-level-fluctuation zone of the Three Gorges Reservoir area. Following 20 d of different treatments, recovery was monitored for 10 d (until day 30) under the control condition.

Physico-chemical properties of floodwater

Physico-chemical properties of the floodwater were checked in the morning (1000 – 1200 h) on day 0, 1, 7, 13 and 19 during the submergence treatment. Water samples were randomly taken from three out of six submergence tanks. The inorganic carbon concentrations, including dissolved CO_2, HCO_3^- and CO_3^{2-}, were estimated by using the titration method described by Klee (1998). The level of dissolved O_2 was measured with an O_2 electrode (AM 39, Sensortechnik Meinsberg, Germany). Temperature and pH were also determined to calculate the CO_2 and O_2 levels. The mean values of these parameters are summarised in Table 1. Occasionally, the sampling procedure might have reoxygenated the floodwater to result in overestimation of the O_2 concentration.

TABLE 1. Physico-chemical properties of floodwater in submergence tanks

Parameter	Day 0	Day 1	Day 7	Day 13	Day 19
Temperature (^{o}C)	18.7	19.0	21.1	20.6	21
pH	6.7	6.7	6.7	6.1	6.2
HCO_3^- (μmol L^{-1})	425 ± 14	427 ± 16	328 ± 12	211 ± 54	257 ± 56
CO_2 (μmol L^{-1})	191 ± 4	189 ± 8	127 ± 8	296 ± 57	274 ± 25
O_2 (μmol L^{-1})	158 ± 1	151 ± 4	225 ± 6	203 ± 9	166 ± 7

All values are means (± s.e.) of three individual measurements from three flooding tanks per treatment. The amounts of CO_3^{2-} detected in the floodwater were below 1 μmol/L in all treatments.

Measurements of the maximal quantum yield of photosystem II

The maximal quantum yield of photosystem II (Fv/Fm) was determined by chlorophyll *a* fluorescence measurements. Measurements were performed in the glasshouse in the morning (0900 – 1000 h) every other day during the treatments (from day 0 to day 20; data not shown) and everyday during the recovery (from day 20 to day 30). For the submerged plants, no measurement was done during the submergence treatment. The fluorescence yield was measured on the youngest fully-expanded leaves (i.e. mature leaves closest to the growth zone of the shoot) of potted plants by using a Handy PEA instrument (Hansatech Instruments, King's Lynn, UK). The youngest fully-expanded leaves changed as the control plants continued to grow and form new leaves during the 30-d experiment, while those of the de-shaded and de-submerged plants were the same leaves throughout the experiment. The maximal and minimal fluorescence intensity of dark-adapted leaves (Fm and Fo, respectively) were determined after 30-min dark adaptation by 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. The Fv/Fm was calculated as Fv/Fm = (Fm – Fo)/Fm.

Leaf and root growth analyses

Leaf and root growth were analysed for plants growing in rhizotrons. Measurements were performed every other day during the treatments (from day 0 to day 20; data not shown) and everyday during the recovery (from day 20 to day 30). For the submerged plants, no measurement was performed during the submergence treatment.

Length and width of each leaf for *A. philoxeroides*, or length of each leaf for *H. altissima*, were measured manually with a ruler and leaf area was calculated according to the following equations:

$$\text{Leaf area } [\text{cm}^2] = \text{length [cm]} \times \text{width [cm]} \times f + b \quad \text{(Eqn. 1)}$$

$$\text{Leaf area } [\text{cm}^2] = \text{length [cm]} \times f - b \quad \text{(Eqn. 2)}$$

where *f* and *b* are geometric factors obtained by calibration with > 50 leaves of different size which were scanned by a conventional desktop scanner (scannersystem STD 1600, Regent Instruments Inc., Quebec, Canada) to determine the area. Calibration was done separately for each treatment and species. The *f* values used for the control, de-shaded and de-submerged plants were: 0.6476, 0.6344 and 0.6505 for *A. philoxeroides* and 0.486, 0.435 and 0.4655 for *H. altissima*, respectively. The b values used for the control, de-shaded and de-submerged plants were: 0.2602, 0.2076 and 0.1483 for *A. philoxeroides* and 0.9309, 0.7584 and 1.0408 for *H. altissima*, respectively. The total leaf area of each plant was calculated by adding up the area of all leaves.

The rhizotrons were placed in an angle of 45°, with the transparent plexiglass surfaces covered with black plastic sheets to prevent light penetration. A preliminary experiment showed that most roots (> 90%) grew along the plexiglass/soil surface facing downward. Images of the root system growing along the plexiglass/soil surface (Fig. 1A – C) were taken with a SLR camera (D400, Canon; resolution 3888 × 2592), with a spatial resolution of ca. 5.4 pixels mm^{-1}. The pixel number of the total projected (visible) root area was determined with the algorithms and segmentation method described by Walter *et al*. (2007). Roots (white pixels) were automatically distinguished from the soil (dark pixels) to obtain a mask image for each picture (Fig. 1D – F). Noises in the segmentation masks, resulting from white soil particles or water bubbles, were deleted manually by a custom-made 'rubber' tool. Finally, the number of white pixels was converted into the visible root area (cm^2). The projected root area was determined for the whole root system as well as separately for roots distributed in three soil layers (top, 0 – 20 cm; middle, 20 – 40 cm; bottom, 40 – 60 cm; Fig. 1D, E).

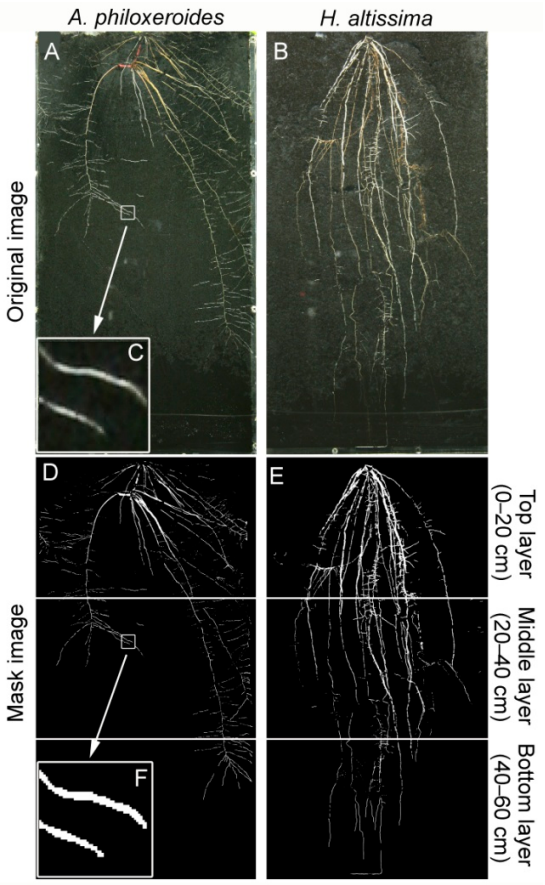

Fig. 1. Roots of *A. philoxeroides* and *H. altissima* growing along the plexiglass/soil surface in rhizotrons.

Original images (A – C) and the corresponding segmented masks (D – F) of projected root area. The square regions indicated in (A) and (D) are magnified in (C) and (F), respectively. The mask images are divided into three soil layers: top (0 – 20 cm), middle (20 – 40 cm) and bottom (40 – 60 cm) layer. The images shown are from de-submerged plants after 10 d of recovery (day 30).

For both leaves and roots, relative growth rate (RGR, % d^{-1}) was calculated as:

$$\text{RGR}\ [\%\ d^{-1}] = 1/dt \times \ln (A_2 / A_1) \qquad \text{(Eqn. 3)}$$

where A_1 and A_2 are the total leaf area or the projected root area at time 1 and 2, respectively, and dt is the number of days between the time 1 and 2.

Dry weight measurements

Data of dry weight were collected at the beginning (day 0, n = 8) and at the end of the treatments (day 20, n = 4) as well as after 10 d of recovery (day 30, n = 4). At each sampling point, potted plants were harvested and put in an oven at 75°C until a constant weight was reached. Plant materials were divided into leaves, stems/culms and roots.

Carbohydrate analysis

Samples for carbohydrate analysis were harvested in the morning (1000 – 1100 h) from potted plants before (day 0) and at the end of the shading or submergence treatment (day 20) as well as during the 10-d recovery. The youngest fully-expanded leaves were collected from the control, de-shaded and de-submerged plants at each sampling time; these leaves were equivalent to the leaves used for the chlorophyll *a* fluorescence measurements. To compare the effects of the three treatments in leaves of the same age, basal leaves of the control plants ('control basal') were also collected during the recovery period; these were the youngest fully-expanded leaves on day 0, thus at the same age as the sample leaves of the de-shaded and de-submerged plants. The internode just below each sample leaf and segments of root tissues (approx. 20 mg) at about 3 cm from the root base were also collected from the same plants. All samples were frozen in liquid nitrogen immediately after harvesting and stored at -80^{o}C until extraction.

Soluble sugars were extracted from the samples according to the method described by Czech *et al.* (2009). Concentrations of glucose, fructose and sucrose were determined by the coupled enzymatic assay (Jones *et al.*, 1977) using a 96-well microplate photometer (ht II, Anthos Mikrosysteme, Krefeld, Germany). Fructan concentrations were measured by using the Megazyme Fructan HK Assay kit (AOAC Method 999.03 and AACC Method 32.32; Megazyme International, Wicklow, Ireland) following the instruction by the company. After extraction of soluble sugars, the remaining plant materials were homogenised and prepared for starch analysis according to Czech *et al.* (2009). The homogenate samples were autoclaved for 90 min at 120^{o}C and 0.1 MPa, then an aliquot (100 µL) was incubated with 1.4 U amyloglucosidase and 1 U α-amylase in 400 µL 50 mM Na-acetate buffer (pH 4.9) for 16 h at 37^{o}C. Then, starch concentrations were determined as glucose by the protocol of Jones *et al.* (1977). Carbohydrate concentrations were expressed relative to the fresh weight (FW) of the samples (µmol g^{-1} FW).

Statistical data analysis

Dry weight data were checked for normal distribution, independence and homogeneity between populations by using SPSS (SPSS Inc., Chicago, IL, USA). The data were statistically tested for differences between treatments (having the same sampling size) by

analysis of variance (one-way ANOVA) or for differences between sampling times (having different sampling sizes) by Kruskal-Wallis test (both in SigmaStat 2.0).

RESULTS

Maximal quantum yield of photosystem II

The maximal quantum yield of photosystem II (Fv/Fm) stayed high in the youngest fully-expanded leaves of the control plants for both *A. philoxeroides* and *H. altissima* throughout the experiment (Fig. 2), suggesting that the control condition did not cause severe photoinhibitory damage. The shading treatment quickly increased Fv/Fm to the maximal level in both species, followed by a minor gradual decrease (data not shown). Removal of the shade cloth on day 20 resulted in a further decrease in Fv/Fm, which was more pronounced in *H. altissima* than *A. philoxeroides* (Fig. 2). During the subsequent recovery period, Fv/Fm was similarly high in

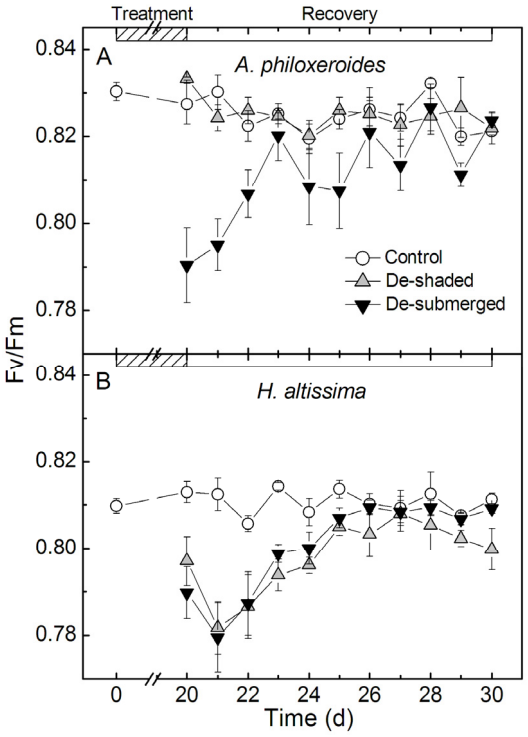

Fig. 2. Changes in the maximal quantum yield of photosystem II (Fv/Fm) measured in leaves of *A. philoxeroides* (A) and *H. altissima* (B) during the 10-d recovery following 20 d of the shading or submergence treatment.
Plants staying under the growth condition (Control, white circle); plants covered with a shade cloth during day 0 – 20 (De-shaded, grey triangle); plants submerged during day 0 – 20 (De-submerged, black inverted triangle). All plants were grown under the control condition until day 0. The shaded plants and the submerged plants were brought back to the control condition on day 20. The youngest fully-expanded leaves were used for all measurements; the youngest fully-expanded leaves changed as the control plants continued to grow and form new leaves, while they were the same leaves for the de-shaded plants and the de-submerged plants throughout the experiment. Symbols are mean values (± s.e., $n = 4$).

the de-shaded and control plants for *A. philoxeroides* while it did not reach the control level until day 25 in the de-shaded plants of *H. altissima*. The Fv/Fm of the de-submerged plants measured on day 20 was significantly reduced in both species. Thereafter, the recovery patterns of Fv/Fm were remarkably similar in the de-submerged and de-shaded plants of *H. altissima*. The de-submerged plants of *A. philoxeroides* showed faster Fv/Fm recovery than the de-submerged and de-shaded plants of *H. altissima*, reaching the level of the control plants already on day 23. These results are largely in agreement with the observations in our previous study, in which the two species were subjected to a longer treatment (30 d) with shading or submergence (Luo *et al*., 2009). Only the de-submerged plants of *H. altissima* exhibited lower Fv/Fm values in the present study compared with the previous study, in which loss of mature leaves during the submergence treatment had been slightly more pronounced.

Leaf and root growth

Growth of leaves and roots was continuously monitored in the plants growing in rhizotrons. Relative growth rate (RGR) calculated from the total leaf area and projected root area gradually decreased in the control plants of the two species with increasing plant size (Figs 3 and 4A, E). The root RGR of *A. philoxeroides* was generally twice as high as that of *H. altissima* despite rather similar leaf RGR.

Shading rapidly suppressed the leaf RGR to about 1 – 2% d^{-1} in both species (Fig. 3). After return to the control condition, the leaf RGR of the de-shaded plants recovered to > 4% d^{-1} within 2 d for *A. philoxeroides* while only weak recovery was observed in *H. altissima*. From day 20 to day 21 the leaf RGR of the de-submerged plants was as low as that of the de-shaded plants in both species. Unlike the contrasting recovery patterns found in the de-shaded plants, however, the de-submerged plants of the two species showed similarly fast and strong recovery of the leaf RGR. The de-submerged plants of *A. philoxeroides* showed the first sign of strong leaf growth recovery on day 23 (Fig. 3A), coinciding with the full recovery of Fv/Fm (Fig. 2A). In the de-submerged plants of *H. altissima*, the leaf RGR started to increase on day 22 (Fig. 3B), i.e. 3 d earlier than the full recovery of Fv/Fm (Fig. 2B). Seven days after returning to the control condition, the de-shaded and de-submerged plants of both species exhibited comparable leaf RGR values.

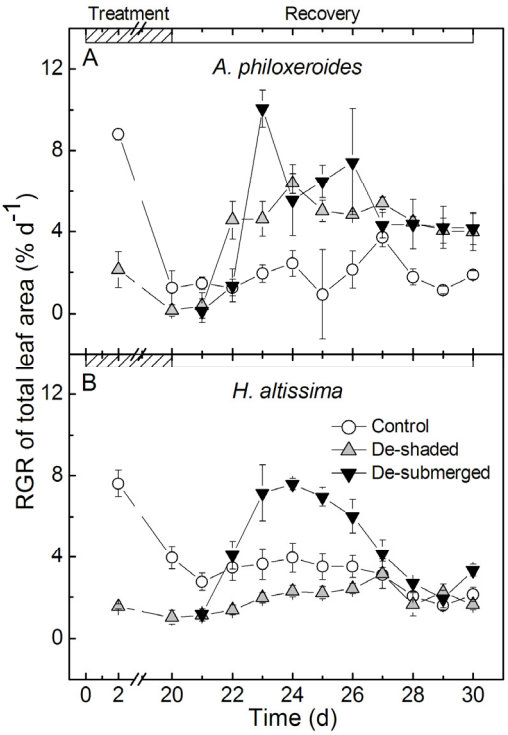

Fig. 3. Changes in relative growth rate (RGR) of the total leaf area of *A. philoxeroides* (A) and *H. altissima* (B) during the 10-d recovery following 20 d of the shading or submergence treatment.

Control plants (white circle); De-shaded plants (grey triangle); De-submerged plants (black inverted triangle). Measurements for the submerged plants were started at the end of the submergence treatment. Symbols are mean values (± s.e., $n = 3$).

Shading inhibited root growth more severely than leaf growth, resulting in zero RGR for most of the treatment period (data not shown). Then, root growth recovery started in the de-shaded plants on day 23 – 24 for both species (Fig. 4A, E). Compared with the de-shaded plants, root RGR recovery in the de-submerged plants was delayed by 1 – 2 d for *A. philoxeroides*, but not *H. altissima*. Comparing leaf and root growth recovery, we observed a delay of 1 – 2 d for root growth recovery, except in the de-shaded plants of *H. altissima* which did not show strong leaf growth recovery (Fig. 3B). In contrast to the similar leaf RGR values found in the de-shaded and de-submerged plants later in the recovery period (Fig. 3; day 27 – 30), the de-submerged plants of both species maintained higher root RGR than the de-shaded plants until the end of the experiment (Fig. 4A, E).

The root system of *A. philoxeroides* had a few tap roots and many horizontally growing secondary roots (Fig. 1A), most of which were distributed in the upper two soil layers when the experiment was started (0 – 20 cm, Fig. 4B; 20 – 40 cm, Fig. 4C); this type of root system can encompass a large volume of substrate in shallow soil (Canadell and Zedler, 1994; Nagel *et al*., 2009). While the control plants could grow roots down to the bottom soil layer (40 – 60 cm) by day 10, only two de-shaded plants and one de-submerged plant reached this depth later

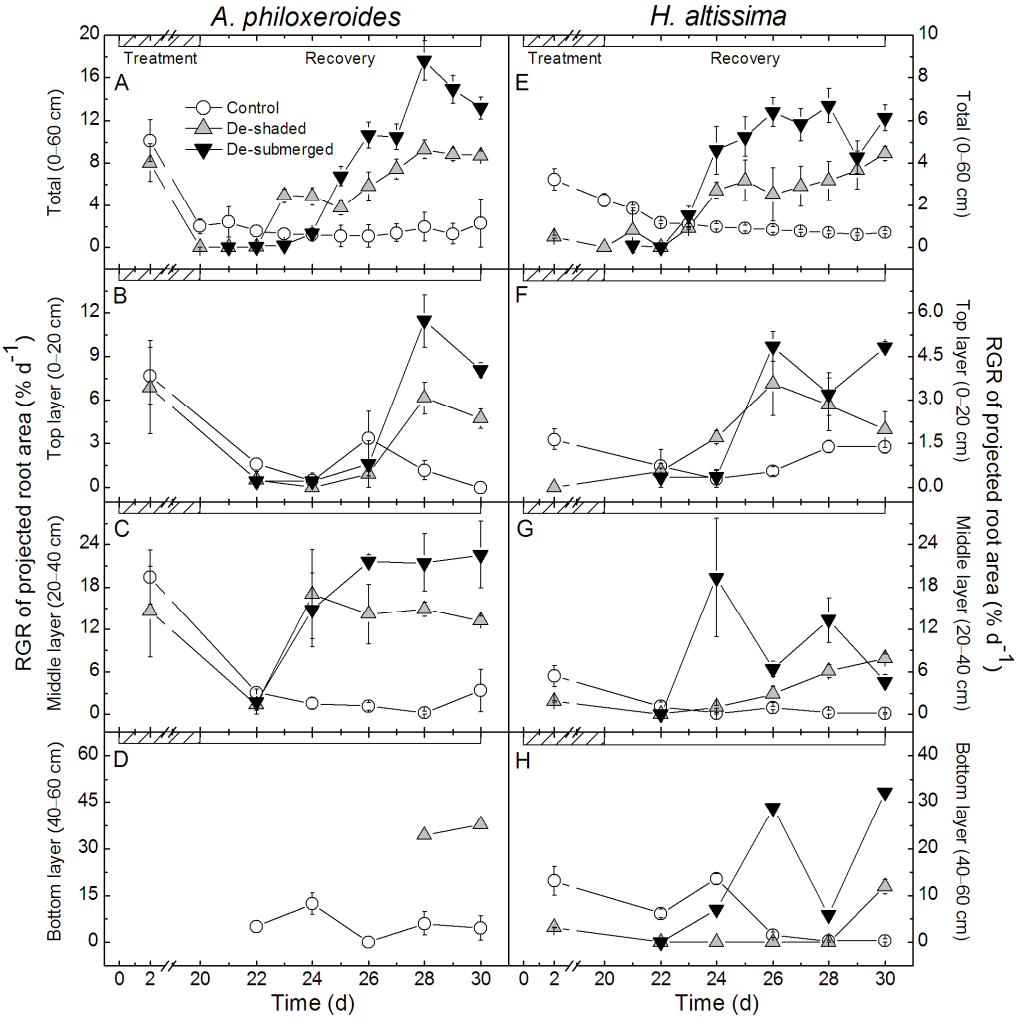

Fig. 4. Changes in relative growth rate (RGR) of the projected root area of *A. philoxeroides* (A – D) and *H. altissima* (E – H) during the 10-d recovery following 20 d of the shading or submergence treatment.

The RGR was calculated from the total projected root area (A, E) as well as from the projected root area in the top (0 – 20 cm), middle (20 – 40 cm) and bottom (40 – 60 cm) soil layers (B – D, F – H). Measurements for the submerged plants were started at the end of the submergence treatment. For abbreviations of the samples, see legend to Fig. 3. Symbols are mean values of three plants (± s.e.) except for the de-shaded plants of *A. philoxeroides* (D) and the de-submerged plants of *H. altissima* (H) in the bottom soil layer, which are means of two plants.

in the recovery period due to strong growth inhibition during the 20-d treatments (Fig. 4D, data of the single de-submerged plant are not shown). In comparison, the root system of the grass *H. altissima* was characterised by vertically growing adventitious roots with short secondary roots (Fig. 1B), as found in rice plants (Kato and Okami, 2009); this type of root system is advantageous to explore substrate in deeper soil. For *H. altissima* all plants but one had roots in the bottom soil layer from the beginning of the experiment (Fig. 4H).

Root growth was also analysed in the three soil layers separately (Fig. 4B – D, F – H). The root RGR of the control plants was the highest in the bottom layer and the lowest in the top layer because the relative amount of growing roots was greater in the apical region than in the basal region of the root system. Root growth recovery of both de-shaded and de-submerged plants started from the deeper soil layers for *A. philoxeroides* (Fig. 4B – D), whereas root growth in the de-shaded plants of *H. altissima* did not recover in the bottom layer until day 30 (Fig. 4H). Although the separate analysis in different soil layers resulted in strong day-to-day variations of RGR in the de-submerged plants of *H. altissima*, these plants did not show prolonged suppression of root growth in the bottom soil layer.

Dry mass accumulation

Growth recovery was also examined by measuring the dry weight of leaves, stems/culms and roots on day 0, day 20 and day 30. The dry weight of leaves, stems and roots increased by approx. 22, 39 and 33 times, respectively, in the control plants of *A. philoxeroides* between day 0 and day 30 (Fig. 5A – C) while the corresponding increase in *H. altissima* was nine, 15 and six times (Fig. 5D – F). There was no significant dry mass accumulation in these two species during the 20-d shading or submergence treatment, except in the submerged plants of *A. philoxeroides* in which a minor increase in the stem dry weight was observed (Fig. 5B) together with a small decrease in the leaf dry weight (Fig. 5A). During the recovery of 10 d, dry weight of the de-shaded plants of *A. philoxeroides* increased in leaves, stems and roots by 400, 260 and 100%, respectively, while the corresponding increase was much smaller in the de-submerged plants (180, 50 and 40%) (Fig. 5A – C). Note that the recovery of the leaf RGR was comparable in the de-shaded and de-submerged plants of *A. philoxeroides* (Fig. 3A), and that of the root RGR was even higher in the de-submerged plants than in the de-shaded plants (Fig. 4A). For *H. altissima*, recovery of dry mass accumulation was rather similar in the de-

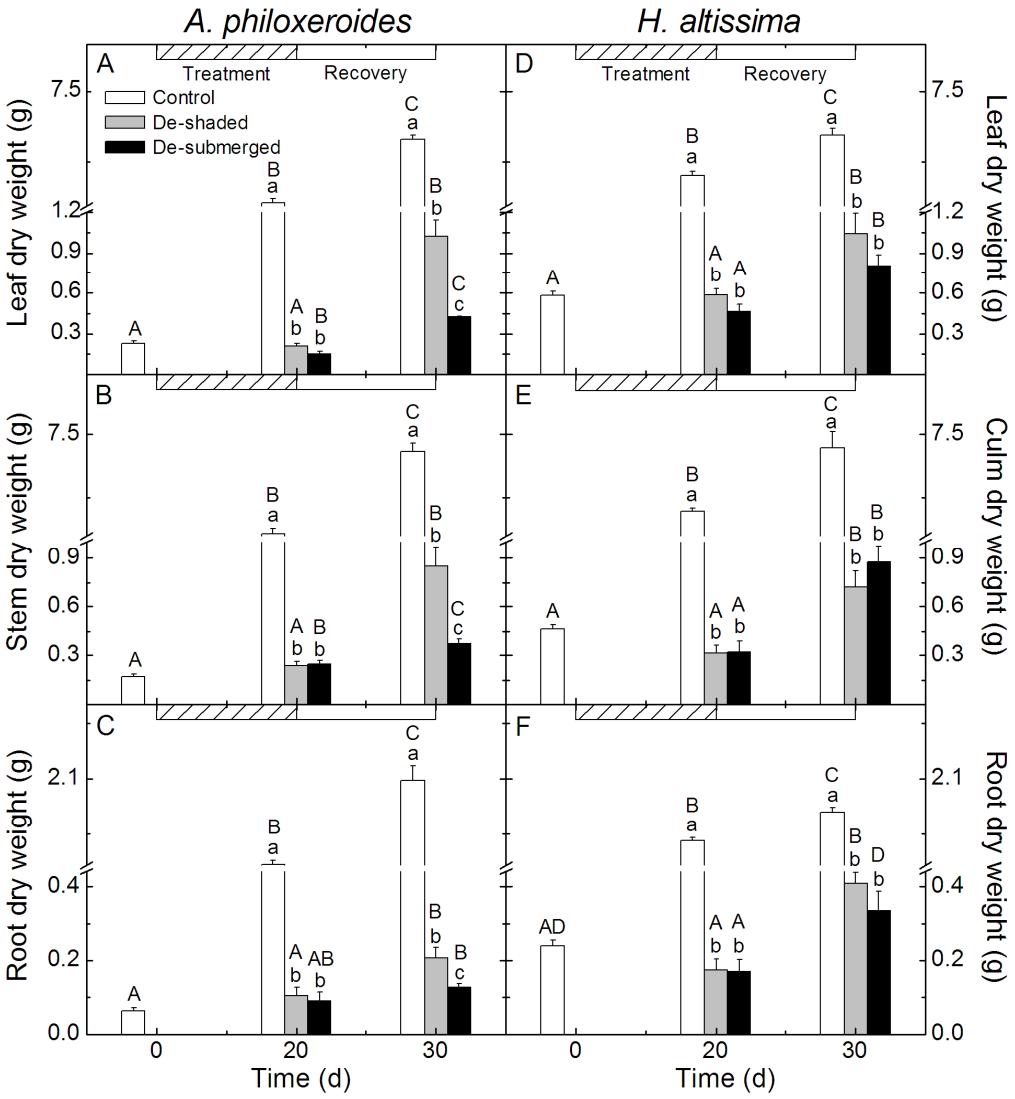

Fig. 5. Dry weight of leaves (A, D), stems/culms (B, E) and roots (C, F) of *A. philoxeroides* (A – C) and *H. altissima* (D – F).

Data were collected before (day 0, n = 8) and at the end of the shading or the submergence treatment (day 20, n = 4) as well as following the subsequent 10-d recovery (day 30, n = 4). Control plants (white bar); De-shaded plants (grey bar); De-submerged plants (black bar). Error bars show s.e. Different lower-case letters indicate statistically significant differences (P < 0.05 by one-way ANOVA) between the treatments at each time point. Different upper-case letters indicate statistically significant differences (P < 0.05 by Kruskal-Wallis test) between the measurement time points for each treatment.

shaded and de-submerged plants (Fig. 5D – F), with an increase of 80, 130 and 130% in leaves, culms and roots of the de-shaded plants and 70, 170 and 100% in the de-submerged plants, respectively. This picture also contrasts with the higher leaf and root RGR found in the de-submerged plants compared with the de-shaded plants of this species (Figs 3B and 4E).

In terms of the relative dry mass increase, *A. philoxeroides* was investing more in stems and roots than in leaves under the control condition. The situation was reversed during the 10-d recovery period following de-shading or de-submergence; the largest relative increase was found in leaves and the smallest in roots. Especially, dry mass accumulation was strongly suppressed in stems of the de-submerged plants during the recovery. The plants of *H. altissima* always showed the highest relative dry mass investment in culms. De-shading and de-submergence also affected the investment of this species in leaves and roots during the recovery, yet in an opposite direction to what were found in *A. philoxeroides*: a greater relative increase for leaves than for roots in the control plants and *vice versa* in the de-shaded and de-submerged plants.

Carbohydrate concentrations

Carbohydrate concentrations were analysed in leaves, stems/culms and roots at the beginning and at the end of the treatments and during the recovery period (Figs 6 – 8). The youngest fully-expanded leaves, similar to those used for the fluorescence analysis (Fig. 2), were harvested in the morning. Data from the basal leaves of the control plants, corresponding to the youngest fully-expanded leaves on day 0 and hence as old as the sample leaves of the de-shaded and de-submerged plants, are also shown for comparison.

Generally, the leaf carbohydrate concentration was much higher in *A. philoxeroides* than in *H. altissima* (Fig. 6). The basal control leaves of *A. philoxeroides* contained large amounts of hexose (glucose + fructose) and starch, in addition to smaller amounts of sucrose and fructan (Fig. 6A – D). After 20 d of shading or submergence, the sample leaves were depleted of these sugars and starch in *A. philoxeroides*. The concentrations of hexose and sucrose increased strongly and transiently in the de-shaded leaves of this species on day 21, while only sucrose showed a similar but less pronounced response in the de-submerged leaves. In comparison, leaves of *H. altissima* retained substantial amounts of sucrose after 20 d of submergence (Fig. 6E – H). A sharp decrease in sucrose was observed in these leaves on day 21,

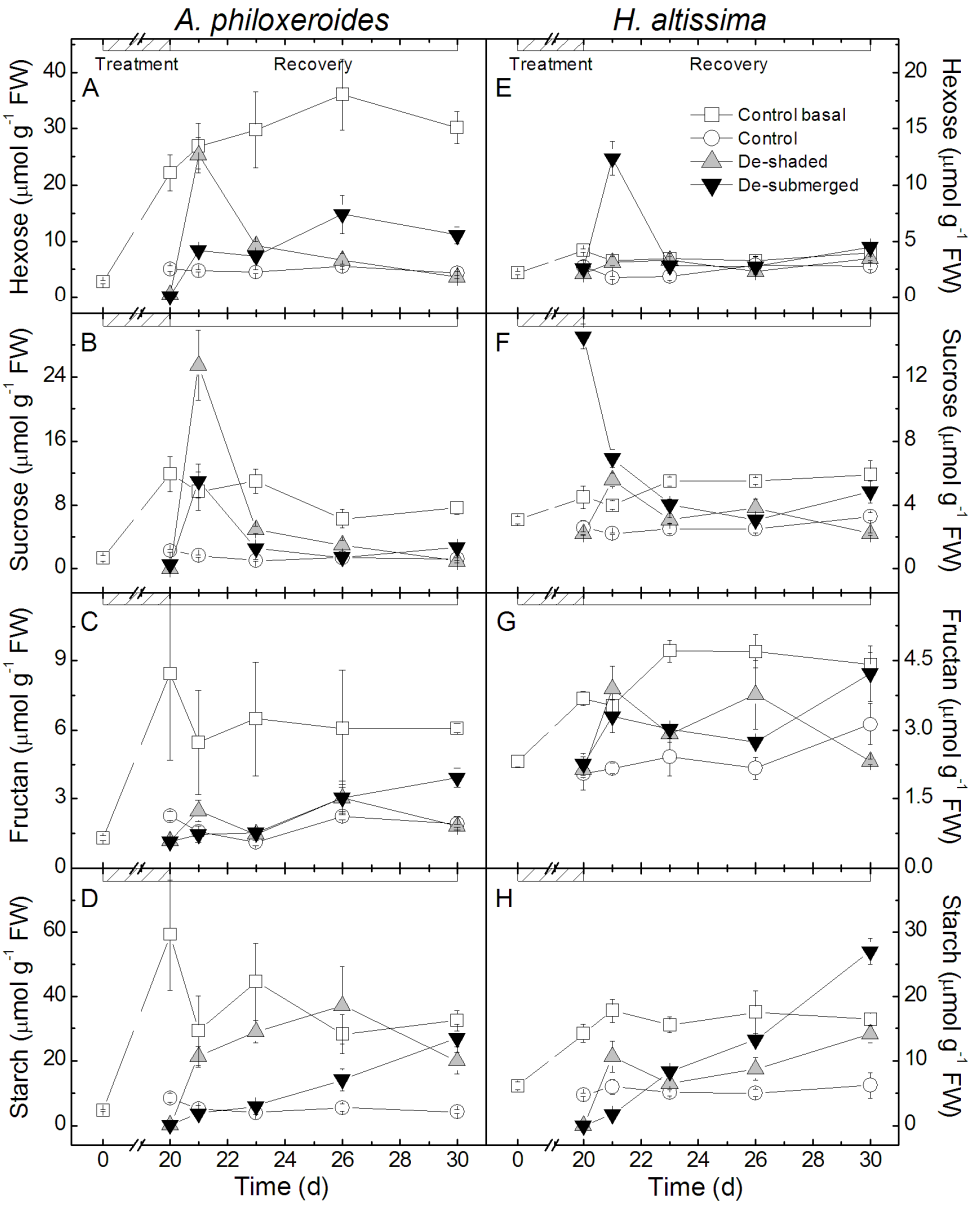

Fig. 6. Concentrations of soluble sugars (A – C, E – G) and starch (D, H) in leaves of *A. philoxeroides* (A – D) and *H. altissima* (E – H) before (day 0) and at the end of the shading or submergence treatment (day 20) as well as during the subsequent 10-d recovery.

Carbohydrate concentrations were analysed in the youngest fully-expanded leaves of the Control (white circle), De-shaded (grey triangle) and De-submerged plants (black inverted triangle) at each time point. Also shown are data from basal leaves of the control plants (Control basal, white square); these were the youngest fully-expanded leaves on day 0, thus at the same age as the sample leaves of the de-shaded plants and the de-submerged plants during the recovery period. Starch concentrations are given in equivalent amounts of glucose. Symbols are mean values (± s.e., $n = 4$).

together with an increase in hexose (Fig. 6E, F). Such symptoms were not observed in the de-shaded leaves. Notably, the transient sugar peaks preceded the strong recovery of leaf growth, another process which was lacking in the de-shaded plants of *H. altissima* (Fig. 3B). The starch concentration recovered in the sample leaves of the two species in the same way (Fig. 6D, H); the largest increase occurred on day 21 in the de-shaded leaves, while the increase was slower but continuous in the de-submerged leaves. On day 30 the de-shaded and de-submerged leaves had similar starch levels in *A. philoxeroides*, whereas the de-submerged leaves had more starch than the de-shaded ones for *H. altissima*.

The sugar levels in the internodes — just below the leaves used for the analysis in Fig. 6 — were far greater in *H. altissima* than *A. philoxeroides* (Fig. 7). In particular, the control and basal control samples of *H. altissima* had large amounts of hexose and sucrose, respectively (Fig. 7E, F). The carbohydrate storage was almost exhausted in the shaded and submerged samples of *A. philoxeroides* on day 20 (Fig. 7A – D). The same was true for the shaded samples of *H. altissima* (Fig. 7E – H) while the submerged samples still contained some sucrose (Fig. 7F). Unlike in leaves, no transient change in sugars was observed in the internode samples of *A. philoxeroides* (Fig. 7A, B) or *H. altissima* (Fig. 7E, F) on day 21. The two species showed similar recovery patterns of sugar and starch concentrations in the internodes, i.e. faster in the de-shaded samples than in the de-submerged samples. However, the hexose levels of the de-submerged samples exceeded those of the de-shaded samples for *H. altissima* by day 26, which is in line with the slightly higher culm dry weight found for the de-submerged plants on day 30 (Fig. 5E).

The carbohydrate analysis was also conducted for root segments collected at ca. 3 cm from the root base (Fig. 8). No root sample was taken from apical regions (closer to the growing zone) due to difficulty in sampling. The root carbohydrate concentration was much higher in *A. philoxeroides* (Fig. 8A – D) than in *H. altissima* (Fig. 8E – H). While starch was the major form of carbohydrate in the basal leaves of *A. philoxeroides* (Fig. 6D), the basal roots of this species accumulated substantial amounts of fructan (Fig. 8C) along with hexose and sucrose (Fig. 8A, B). Again, the basal roots of the two species contained little carbohydrate after the shading or submergence treatment; unlike in leaves and internodes (Figs 6F and 7F), however, the submerged plants of *H. altissima* retained little sucrose in roots (Fig. 8F). The carbohydrate levels increased in the de-shaded roots of *A. philoxeroides* during the

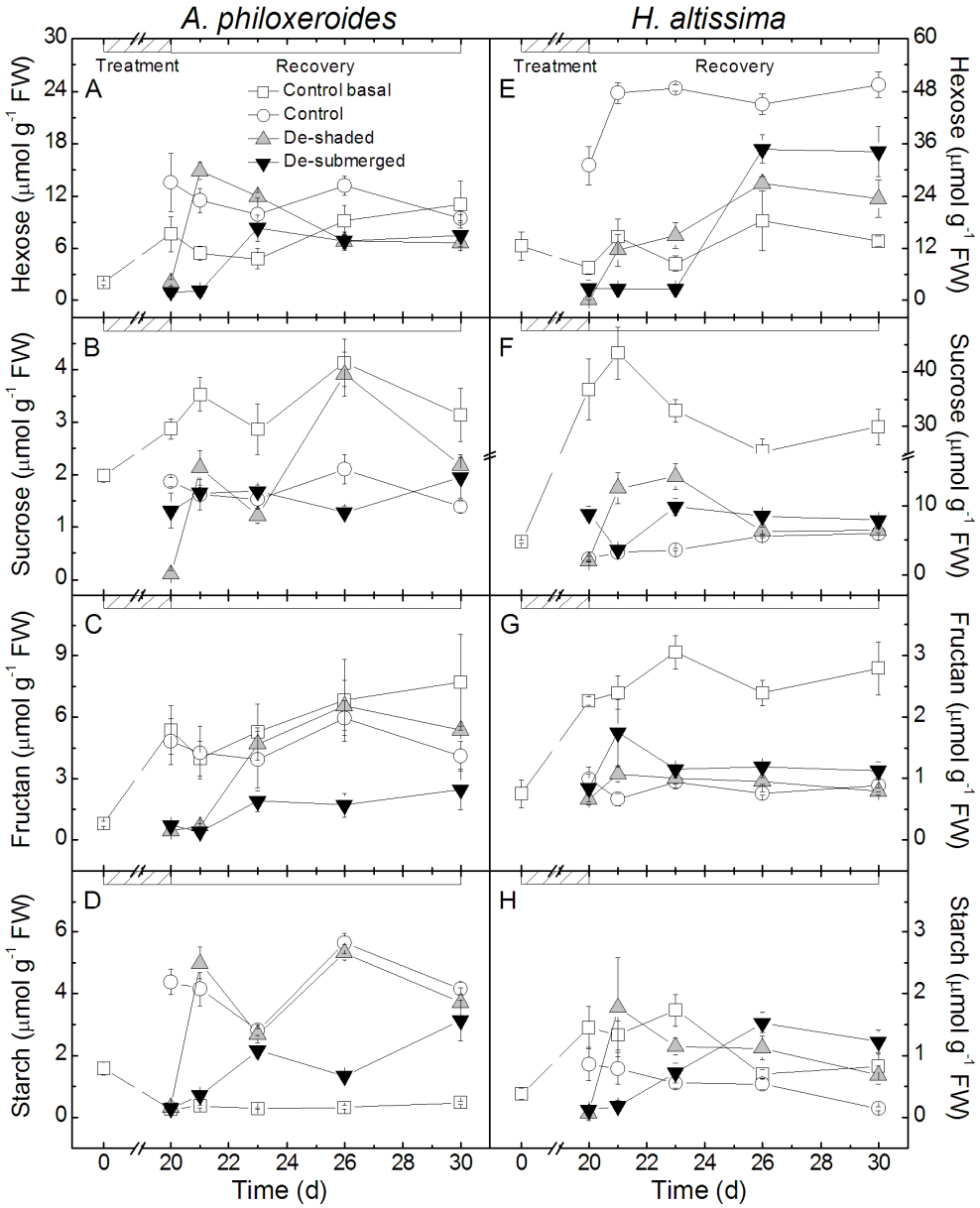

Fig. 7. Concentrations of soluble sugars (A – C, E – G) and starch (D, H) in stems of *A. philoxeroides* (A – D) and culms of *H. altissima* (E – H) before (day 0) and at the end of the shading or submergence treatment (day 20) as well as during the subsequent 10-d recovery. The internode just below each sample leaf in the experiment of Fig. 6 was harvested for the analysis. Starch concentrations are given in equivalent amounts of glucose. For abbreviations of the samples, see legend to Fig. 6. Symbols are mean values (± s.e., $n = 4$).

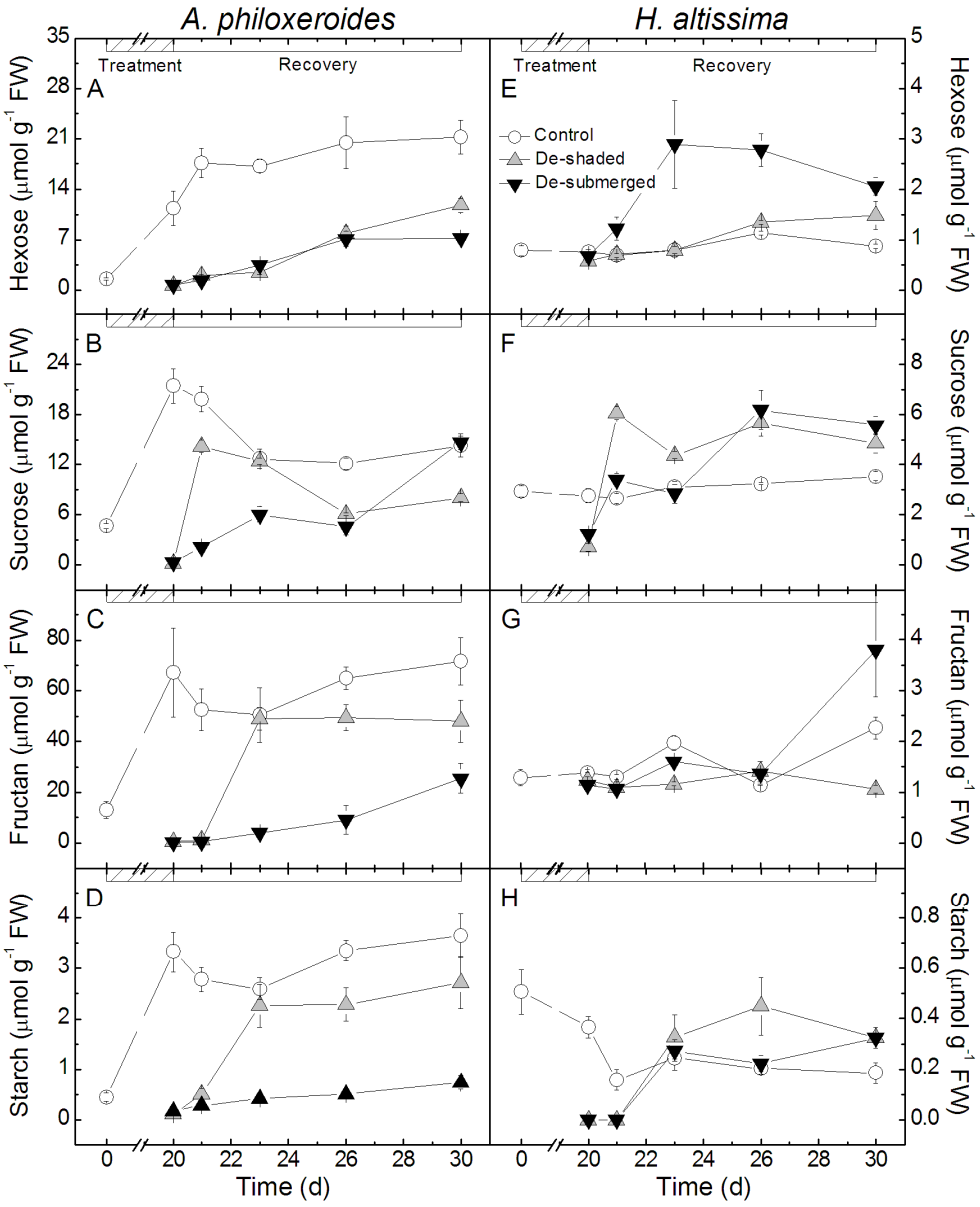

Fig. 8. Concentrations of soluble sugars (A – C, E – G) and starch (D, H) in roots of *A. philoxeroides* (A – D) and *H. altissima* (E – H) before (day 0) and at the end of the shading or submergence treatment (day 20) as well as during the subsequent 10-d recovery.

Segments of root tissues (about 20 mg) were harvested at *c*. 3 cm from the root base for all three treatments. Control plants (white circle); De-shaded plants (grey triangle); De-submerged plants (black inverted triangle). Starch concentrations are given in equivalent amounts of glucose. Symbols are mean values (± s.e., $n = 4$).

recovery period, starting from sucrose followed by fructan and starch, then finally hexose (Fig. 8A – D). In comparison, carbohydrate accumulation was retarded in the de-submerged roots of *A. philoxeroides*, consistent with the smaller increase in the root dry weight of these plants during the recovery period (Fig. 5C). Although the changes in the root carbohydrate concentration were less dramatic in *H. altissima*, the recovery was not much retarded, or even faster for hexose, in the de-submerged roots compared with the de-shaded roots (Fig. 8E – H).

DISCUSSION

The major findings in the de-shaded and de-submerged plants of *A. philoxeroides* and *H. altissima* concerning the status at the end of, and the recovery patterns shortly after, the 20-d treatments are summarized in Table 2. Below we discuss these results, focusing on the behaviour before and after de-submergence, the relationships between the recovery of photosynthesis, carbohydrate accumulation and growth, as well as the biomass allocation between shoots and roots.

Responses of A. philoxeroides *and* H. altissima *during submergence*

Submergence arrested both above- and belowground dry mass accumulation in *A. philoxeroides* and *H. altissima* (Fig. 5). Similar responses were also found in the shaded plants, underlining the sensitivity of growth processes to severe light limitation and starvation. The growth inhibition during the shading treatment was more pronounced for roots than for leaves (data not shown), presumably because of high susceptibility of root growth to energy deprivation (Nagel *et al*., 2006, 2009) and/or importance of maintaining leaf area growth under low irradiance to augment light capture (Poorter and Nagel, 2000; Markesteijn and Poorter, 2009).

At the end of the 20-d submergence, the maximal PSII efficiency was reduced in leaves (Fig. 2) and the carbohydrate storage of the sample tissues was largely depleted for both species (Figs 6 – 8), except in leaves and culms of *H. altissima* which retained ≥ 10 μmol sucrose g^{-1} FW. Under energy crisis, such as during our submergence and shading treatments, remobilization of stored carbohydrate plays a vital role in providing energy needed for cell and

TABLE 2. Summary of the responses of photosynthesis, growth and carbohydrate accumulation in de-shaded and de-submerged plants

	A. philoxeroides		*H. altissima*	
	De-shaded	De-submerged	De-shaded	De-submerged
End of the treatments				
Fv/Fm	high	reduced	as Control	reduced
Leaf RGR	1 – 2% d^{-1}	1 – 2% d^{-1}	1 – 2% d^{-1}	1 – 2% d^{-1}
Root RGR	0% d^{-1}	0% d^{-1}	0% d^{-1}	0% d^{-1}
Dry weight	no change	decrease in leaves, minor increase in stems	no change	no change
Carbohydrate	depleted	depleted	depleted	sucrose accumulation in leaves and culms
Recovery				
Fv/Fm	as Control	day 23	day 25	day 25
Leaf RGR	day 22	day 23	weak	day 22
Root RGR	day 23	day 24 – 25	day 23 – 24	day 23 – 24
	from apical region	from apical region	retarded in apical region	from apical region
Dry weight increase	largest in leaves	largest in leaves	largest in culms and roots	largest in culms
Carbohydrate	day 21	day 21 in leaves, day 23 in stems and roots	day 21	sucrose hydrolysis, recovery on day 23

organ maintenance as well as stress responses (Greenway and Gibbs, 2003). Anoxia-tolerant plants, like rice, *Acorus calamus* and *Potamogeton pectinatus*, are able to degrade starch under low O_2 conditions by, for example, increasing the activity of α-amylase (Arpagaus and Braendle, 2000; Dixon *et al*., 2006; Magneschi and Perata, 2009). The two species examined in this study remobilized starch and sugars during submergence (Figs 6 – 8), demonstrating their ability to utilise the carbohydrate storage under low O_2, CO_2 and PAR, the conditions often found in deep or turbid floodwater.

The peculiar accumulation of sucrose found in the submerged plants of *H. altissima* (Figs 6F and 7F), but not *A. philoxeroides* (Figs 6B, 7B and 8B), are consistent with the contrasting survival strategies proposed for these plants (Luo *et al*., 2009): the quiescence strategy of *H. altissima*, showing no obvious growth response during submergence, as opposed to the escape strategy of *A. philoxeroides*, showing enhanced stem elongation. In our previous study (Luo *et al.*, 2009), the average stem elongation rate observed in *A. philoxeroides* was three times

higher during submergence (1.28 cm d^{-1}) than after de-submergence (0.37 cm d^{-1}). However, unlike normal growth involving biomass accumulation, the submergence-induced stem elongation in *A. philoxeroides* was accompanied by little biomass accumulation (Fig. 5B). Still, it may have contributed to carbohydrate depletion in the submerged plants of *A. philoxeroides*, as was seen in *Rumex palustris* showing submergence-induced petiole elongation (Groeneveld and Voesenek, 2003). On the other hand, it seems that submergence downregulated energy consumption and carbohydrate catabolism in *H. altissima* (Gibbs and Greenway, 2003; Greenway and Gibbs, 2003), leading to sucrose retention in the submerged plants, but not in the shaded plants (Figs 6F and 7F). Since the PAR in our submergence treatment was extremely low, this sucrose accumulation in leaves and culms of *H. altissima* must have engaged synthesis of sucrose from remobilized carbohydrate.

Recovery of photosynthesis, growth and carbohydrate accumulation after de-submergence

Following de-shading or de-submergence on day 20, recovery of the leaf and root RGR started 1 or 2 d earlier in the de-shaded plants than in the de-submerged plants for *A. philoxeroides* (Figs 3A and 4A). The faster growth recovery of the de-shaded plants was accompanied by high Fv/Fm in their leaves (Fig. 2A) and immediate recovery of carbohydrate accumulation on day 21 (Figs 6 – 8). The lower photosynthetic capacity of the de-submerged plants early in the recovery period (Fig. 2A), presumably caused by structural and/or functional damages to chloroplasts (Panda *et al*., 2006), may have delayed the recovery of carbohydrate accumulation and growth in these plants. With the carbohydrate storage nearly exhausted (Figs 6 – 8), growth recovery of the de-submerged and de-shaded plants of *A. philoxeroides* must have been largely dependent on, and thus limited by, newly synthesized photoassimilates. It is noteworthy that the strong leaf RGR recovery on day 23 coincided with the full recovery of Fv/Fm in the de-submerged plants of this species (Figs 2A and 3A).

Plants grow vegetative organs to maximize the surface area for uptake of the most limiting resources; for example, dry forest plants increase belowground biomass allocation to maximize water uptake, whereas moist forest seedlings have a greater aboveground biomass to maximize light interception (Poorter and Nagel, 2000; Markesteijn and Poorter, 2009). Likewise, defoliated plants of a grass species *Paspalum dilatatum* double the plant height during recovery after de-flooding to increase light capture (Striker *et al*., 2008). In the case of

A. philoxeroides, stem was the only organ elongating underwater (Luo *et al*., 2009), reflecting the urgent need to improve O_2, CO_2 and light availability. Shortly after de-submergence, the relative dry mass increase was the highest in leaves (Fig. 5A), the primary organ for photosynthesis. The leaf RGR increase preceded the root RGR increase by 1 – 2 d in both de-submerged and de-shaded plants (Figs 3A and 4A), suggesting high priority of photosynthesis during the initial recovery period. As with increasing leaf area and thereby improving carbohydrate status, the most limiting resources for these plants would change, inducing a shift of biomass partitioning in the following days (Luo *et al.*, 2009).

A different picture emerged for the recovery of *H. altissima*. The sudden increase in PAR on day 20 similarly inhibited the maximal photosystem II efficiency in both submerged and shaded plants (Fig. 2B), indicating the vulnerability of this species to photoinhibition (Luo *et al.*, 2009). Interestingly, our leaf and root RGR analyses (Figs 3B and 4E) revealed the growth recovery starting earlier than the full recovery of Fv/Fm in the de-submerged plants of *H. altissima*. It seems that the sucrose stored in leaves and culms was rapidly hydrolysed (Figs 6F and 7F) and utilised to promote growth shortly after de-submergence. Besides the high survival rate (90%) recorded previously for *H. altissima* after a long-term submergence of 180 d (Wang *et al.*, 2008), this ability to achieve rapid growth recovery points to the benefit of the quiescence strategy, namely fast generation of new tissues after de-submergence by using the carbohydrate pool retained during submergence (Das *et al*., 2005; Fukao *et al*., 2006; Panda *et al*., 2008; Striker *et al*., 2008; Kawano *et al*., 2009). Also, submergence-induced sucrose retention and its hydrolysis in leaves and culms of *H. altissima* upon de-submergence may provide ATP for protection of cellular components suffering from oxidative stress and for *de novo* lipid synthesis to maintain membrane integrity (Rawyler *et al*., 1999). It is also crucial to prevent depletion of K^+ in leaves, resulting from cytosolic K^+ leak via outward-rectifying K^+ channels activated by reactive oxygen species (Demidchik *et al*., 2010); previous studies have reported a decline in leaf potassium concentration in barley, wheat and corn under flooding conditions (Board, 2008).

The de-shaded plants of *H. altissima* showed neither sucrose retention on day 20 (Figs 6F, 7F and 8F) nor high leaf RGR during the recovery period (Fig. 3B). Despite the quick recovery of carbohydrate accumulation on day 21, these plants maintained low leaf RGR (Fig. 3B) and had also lower root RGR than the de-submerged plants mainly due to the growth

suppression in deeper soil (Fig. 4E – H). Nevertheless, the increase in leaf and root dry weight was comparable in the de-shaded and de-submerged plants of *H. altissima* between day 20 and day 30 (Fig. 5D – F), implying that the dry mass increase in the de-shaded plants relied more on mass accumulation and less on area growth, and *vice versa* for the de-submerged plants. The de-submergence-induced enhancement of leaf and root area growth may serve to compensate leaves and roots lost by injuries during submergence (Fig. 5; Luo *et al.*, 2009). In all treatments, however, the largest dry mass increase was found in culms (Fig. 5E), a storage organ of *H. altissima*. Conservative biomass allocation to storage organs can confer stress tolerance to plants, such as under low irradiance or herbivory attack (Myers and Kitajima, 2007; Imaji and Seiwa, 2010). Our results from *H. altissima* suggest that conservative biomass allocation to storage organs during flood intervals may be a part of submergence tolerance based on the quiescence strategy.

Recovery of root growth

Previous studies in different wetland species have shown that biomass accumulation is more severely suppressed in roots than in shoots following de-flooding (Smethurst *et al*., 2005; Chen and Xie, 2009). This was also the case for *A. philoxeroides* and *H. altissima* in the present study (Fig. 5). The root RGR recovery started 1 – 2 d later than the leaf RGR recovery in the de-submerged plants of both species (Figs 3 and 4A, E), suggesting that the carbohydrate retained or newly synthesized in leaves after de-submergence is initially used for shoot growth. As root growth is closely related to the carbohydrate import from shoot (Farrar *et al.*, 2000; Nagel *et al.*, 2006), its recovery may be confined by suppressed carbohydrate export and phloem loading in mature leaves (Slewinski and Braun, 2010). Additionally, poor sink strength of roots after severe O_2 and energy limitation (Gaynard and Armstrong, 1987; Crawford, 1992; Armstrong *et al*., 1994), even in species having well-developed aerenchyma like *A. philoxeroides* and *H. altissima* (Luo *et al.*, 2009), or effects of toxic components like Mn^{2+}, Fe^{2+} and S^{2-} accumulating in flooded soil (Jackson and Drew, 1984; Greenway *et al.*, 2006) may have delayed the recovery of root growth. Yet, the concurrent root RGR recovery observed in the de-submerged and de-shaded plants of *H. altissima* (Fig. 4E) suggests that the toxic soil components are unlikely to be the cause of the slow root growth recovery at least for this species.

CONCLUDING REMARKS

Stem elongation of the escape strategy did not leave much carbohydrate in *A. philoxeroides* after 20 d of complete submergence, but following de-submergence photosynthesis and carbohydrate accumulation were quickly restored in this species to resume growth. The quiescence strategy conserved some sucrose in shoots of *H. altissima* during submergence, promoting rapid re-growth of these plants shortly after de-submergence before the full recovery of photosynthesis. Submergence tolerance of the two strategies thus seems to entail not only the corresponding regulation of growth, carbohydrate catabolism and energy metabolism during submergence but also coordinated recovery of photosynthesis, growth and carbohydrate partitioning after de-submergence.

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6.3 How do antioxidative defense systems respond to de-submergence in wetland plants having escape and quiescence strategies?

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Own contribution

- Experiments
- Data analysis (with co-authors)
- Preparation of manuscript (with co-authors)

How do antioxidative defense systems respond to de-submergence in wetland plants having escape and quiescence strategies?

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ABSTRACT

Background and Aims Fast recovery after de-submergence requires efficient protection against oxidative injuries. We investigated whether de-submergence responses of antioxidant systems differ in two wetland plants, *Alternanthera philoxeroides* and *Hemarthria altissima*, characterized by 'escape' and 'quiescence' strategies of flood tolerance, respectively.

Methods The antioxidant capacity was assessed in the two species during 10 d of recovery following 20 d of complete submergence (low light + low O_2) or severe shading (low light + ambient O_2). The activities of superoxide dismutase and catalase were measured in leaf and root tissues, along with the concentrations of reduced ascorbate, malondialdehyde and acetaldehyde. In addition, formation of superoxide (O_2^-) and hydrogen peroxide (H_2O_2) was detected in leaves by chemical staining.

Key Results Following de-submergence, plants of *A. philoxeroides* showed a transient burst of acetaldehyde while the concentration of acetaldehyde increased slowly and stayed high in leaves of *H. altissima*. In leaves of *A. philoxeroides* the variations in O_2^- and H_2O_2 correlated with the levels of light and O_2, respectively, whereas neither of the two reactive oxygen species was detected in *H. altissima*. For *A. philoxeroides* the antioxidant capacities changed mostly in leaves during the recovery; for *H. altissima* changes in reduced ascorbate were found in leaves and those of antioxidant enzyme activities in roots. De-submergence caused some lipid peroxidation in leaves of both species.

Conclusions De-submergence responses of the detoxification systems differed between *A. philoxeroides* and *H. altissima*, especially in leaves: dynamic changes were found in *A. philoxeroides* as opposed to little or slow changes in *H. altissima*. Whereas the antioxidant capacities were often strongly influenced by light environments, the toxic compounds and lipid peroxidation indicated harmful effects of changing O_2 concentration which accompanies submergence and de-submergence.

Key words: Acetaldehyde, *Alternanthera philoxeroides*, antioxidant, flooding, *Hemarthria altissima*, lipid peroxidation, reactive oxygen species, shade, submergence, wetland plant.

INTRODUCTION

Complete submergence often causes severe deficiency of light and O_2 to plants (Jackson and Ram, 2003). Under low O_2, energy conversion switches from aerobic to anaerobic respiration (Gibbs and Greenway, 2003). Since production of ATP by anaerobic (ethanolic) fermentation is much less efficient than oxidative phosphorylation, low ATP availability during submergence, leading to, for example, hydrolysis of membrane lipids to free fatty acids (Henzi and Braendle, 1993; Rawyler *et al*., 1999), can become detrimental to cells.

When submerged plants return to terrestrial conditions, concomitant increase in light and O_2 can further intensify the cellular damage in different organs (Sarkar *et al*., 2001; Blokhina *et al*., 2003; Sarkar *et al*., 2006; Santosa *et al*., 2007). Injuries in plants following de-submergence are mainly associated with reactive oxygen species (ROS) and acetaldehyde (Blokhina *et al*., 2000; Boamfa *et al*., 2005). Excessive formation of ROS results in oxidation of lipid membranes, proteins, nucleic acids and carbohydrate (Blokhina *et al*., 2003; Bailey-Serres and Chang, 2005; Santosa *et al*., 2007). Acetaldehyde accumulates in tissues of submerged and de-submerged plants through oxidation of ethanol, the end product of fermentation (Boamfa *et al*., 2005). Detoxification of a ROS hydrogen peroxide (H_2O_2) by the enzyme catalase (CAT) can also form acetaldehyde from ethanol (Boamfa *et al*., 2005). Over-accumulation of acetaldehyde is harmful for organisms because of its tendency to build acetaldehyde-protein and acetaldehyde-DNA adducts (Perata *et al*., 1992; Zhang *et al*., 1997; Boamfa *et al*., 2005). Besides these toxic effects, some ROS and their oxidation products, such as H_2O_2 and aldehydes, are also known as important signalling molecules in plants (Desikan *et al*., 2005; Møller *et al*., 2007; Hill and Bhatnagar, 2009).

A variety of enzymatic and non-enzymatic antioxidants are present in plant cells to prevent or minimize the accumulation of ROS (Blokhina *et al*., 2003); they consist of ROS-scavenging enzymes (e.g. superoxide dismutase SOD, peroxidase and CAT), low-molecular-weight antioxidants (e.g. ascorbate AsA, glutathione and tocopherols) and enzymes for regeneration of antioxidants. The major scavenger of superoxide (O_2^-), another common ROS in plant cells, is SOD (Meloni *et al*., 2003). The O_2^- scavenging by SOD results in formation of H_2O_2 which in turn is detoxified by different reactions: by ascorbate peroxidase in the water-water cycle in chloroplasts, by CAT in peroxisomes, by glutathione peroxidase in cytosol, or by the ascorbate-glutathione cycle in different cell compartments (Mittler, 2002).

Also acetaldehyde can be detoxified by enzymes in plants; acetaldehyde dehydrogenase oxidizes acetaldehyde to far less toxic acetate in cytosol and mitochondria (Meguro *et al*., 2006; Fujiwara *et al*., 2008) and betaine aldehyde dehydrogenase (OsBADH1) in rice can remove acetaldehyde produced by CAT activities in peroxisomes (Mitsuya *et al*., 2009).

Plants having a large detoxification capacity in leaves and roots are more tolerant to different abiotic stress (Bian and Jiang, 2009; Hossain *et al*., 2009; Skutnik and Rychter, 2009). Survival and recovery from anoxia/hypoxia stress also relies in part on the ability of plants to maintain, or upregulate, the defense mechanisms against ROS (Biemelt *et al*., 1998; Blokhina *et al*., 2000; Skutnik and Rychter, 2009) and acetaldehyde (Tsuji *et al*., 2003; Meguro *et al*., 2006; Magneschi and Perata, 2009). In our previous studies, we have reported contrasting recovery behaviour of photosynthesis, carbohydrate accumulation and growth in two flood-tolerant wetland plants, *Alternanthera philoxeroides* and *Hemarthria altissima*, following submergence and de-submergence (Luo *et al.*, 2009, 2010). The responses of *A. philoxeroides* were characterized by stimulated stem elongation during submergence (Luo *et al.*, 2009), which is considered a trait of the "escape" strategy of flood tolerance (Bailey-Serres and Voesenek, 2008), as well as dynamic down- and upregulation of photosynthesis upon submergence and de-submergence, respectively (Luo *et al.*, 2010). In contrast, plants of *H. altissima* did not grow during submergence at all and retained sucrose in shoots; shortly after de-submergence they were able to quickly resume growth by using stored carbohydrate even before full recovery of photosynthesis (Luo *et al.*, 2010). The high survival rates found in these plants after complete submergence of up to 180 d (Wang *et al.*, 2008a, b) and their ability to rapidly recover photosynthesis and growth after de-submergence (Luo *et al.*, 2009, 2010) suggest that they both have efficient defense systems to restrict submergence-induced and de-submergence-induced injuries.

Based on the different responses of photosynthesis, carbohydrate accumulation and growth in *A. philoxeroides* and *H. altissima* during submergence and after de-submergence (Luo *et al.*, 2009, 2010), we hypothesized that detoxification capacities and/or patterns of defense-related responses may also differ between these two species showing the "escape" and "quiescence" strategies of flood tolerance. To test this hypothesis, we measured the changes in antioxidant capacities (activities of SOD and CAT, concentrations of AsA) in leaves and roots of the two species during 10 d of recovery following 20 d of complete submergence. In

parallel, accumulation of acetaldehyde and ROS (O_2^- and H_2O_2) was also examined along with the extent of lipid peroxidation. The light intensities of the submergence treatment were very low (< 10 μmol photons m^{-2} s^{-1}) to mimic typical conditions in deep or turbid floodwater. In order to assess the damage caused by abrupt increase in irradiance (photooxidation), without a change in O_2, some of the plants were subjected to low-light conditions (< 10 μmol photons m^{-2} s^{-1}) during the 20-d treatment.

MATERIALS AND METHODS

Plant materials and growth condition

Alternanthera philoxeroides (Mart.) Griseb. is a perennial C_3 weed which can establish in aquatic, semi-aquatic and terrestrial environments; in terrestrial environments it has a creeping growth habit. *Hemarthria altissima* (Poir.) Stapf & C.E. Hubb. is a perennial stoloniferous C_4 grass which prefers moist soils but are capable of growing in dry land. Both species are distributed in the water-level-fluctuation zone of the Three Gorges Reservoir area in China. For the present study, plants were collected in the Three Gorges Reservoir area and brought to the institute Phytosphäre, Forschungszentrum Jülich, Germany. Plants of *A. philoxeroides* were propagated from cuttings and those of *H. altissima* from tillers. Young plants of *A. philoxeroides* and *H. altissima* were planted in pots (3.8 L, 15 cm diameter × 22 cm, one plant per pot) filled with black peat soil (Graberde; Plantaflor Humus, Vechta, Germany) containing the following nutrients according to the specification by the company: N 120 mg L^{-1}, P_2O_5 120 mg L^{-1}, K_2O 170 mg L^{-1}.

Plants were watered daily during cultivation under semi-controlled conditions in the glasshouse. The air temperature and relative humidity ranged between 20 – 22°C and 40 – 60% during the experimental period, respectively. Illumination in the glasshouse (SON-T AGRO 400, Philips) was automatically turned on when the ambient light intensity outside the glasshouse became < 400 μmol photons m^{-2} s^{-1} (photosynthetically active radiation, PAR) between 0600 and 2200 h local time. Under such conditions, PAR measured at the plant level was 200 – 300 μmol photons m^{-2} s^{-1}.

Experimental design

For the experiments, 128 plants were randomly selected for each species. The design was two species × three treatments × four replicates at each measurement time. Samples were harvested at six time points during the experiments. At the beginning of the experiments (on day 0), plants were divided into three groups and subjected to the following treatments: (1) plants kept under the growth condition in the glasshouse ('control'), (2) plants covered with a neutral shade cloth (without changing the spectral quality of light) in the glasshouse between day 0 and 20 and transferred back to control condition on day 20 ('de-shaded'), and (3) plants fully submerged under 1-m deep water (tap water) in 480-L plastic tanks and covered with a neutral shade cloth in the glasshouse between day 0 and 20 and transferred back to control condition on day 20 ('de-submerged'). In the submergence tanks, shade clothes were spanned at about 1 cm below the water surface to keep submerged plants completely underwater throughout the treatment. The PAR reaching shaded and submerged plants was < 10 μmol photons m^{-2} s^{-1}. These treatments of very low light and complete submergence were applied to simulate the conditions in deep or turbid floodwater typically found in the water-level-fluctuation zone of the Three Gorges Reservoir area. The O_2 concentration in the floodwater ranged between 150 and 220 μmol L^{-1} during the submergence experiment; more detailed information about the physico-chemical properties if floodwater in the submergence tanks can be found in Luo *et al*., (2010) which reports the recovery responses of photosynthesis, carbohydrate accumulation and growth investigated in parallel with the defense responses featured in the present study. No mineral was added to the floodwater during the submergence treatment. Following 20 d of different treatments (on day 20), the shade clothes were removed and the floodwater was drained. Subsequently, recovery was monitored for 10 d (until day 30) under the growth condition in the glasshouse.

Acetaldehyde measurement

Samples for acetaldehyde measurements were harvested in the morning (1000 – 1130 h) before (on day 0) and at the end of the shading or submergence treatment (on day 20) as well as during the 10-d recovery (until day 30). The youngest fully-expanded leaves and whole root systems were collected from control, de-shaded and de-submerged plants at each sampling time. While shaded and submerged plants did not grow during the 20-d treatments, control

plants continued to grow and form new leaves throughout the experiments. To compare the effects of three treatments in leaves of the same age, basal leaves of control plants were also collected during the recovery period; these were the youngest fully-expanded leaves on day 0, thus at the same age as the sample leaves of de-shaded and de-submerged plants. Leaves and roots were frozen in liquid nitrogen and stored at -80^{o}C until analysis. The same samples were also used for measurements of malondialdehyde (MDA) concentration and antioxidant assay.

The analysis of acetaldehyde was performed on a HP 5890 II gas chromatograph (Hewlett Packard, Waldbronn, Germany) equipped with a flame ionization detector (FID). Separation was performed on a fused-silica capillary column FS-CS-624-CB-1.4 (60 m × 0.25 mm, 1.4 µm film thickness; CS-Chromatographie-Service, Langerwehe, Germany) and helium as carrier gas at a flow rate of 1.0 ml min^{-1}. The oven temperature was held at 35^{o}C for 2 min, increased to 240^{o}C in three-step linear gradients (from 35 to 160^{o}C by 4^{o}C min^{-1}, from 160 to 180^{o}C by 5^{o}C min^{-1}, and from 180 to 240^{o}C by 10^{o}C min^{-1}), and held at 240^{o}C for 3 min. A Perkin Elmer HS 40 headspace autosampler (Perkin Elmer, Massachusetts, USA) was used for sampling. The temperature of the needle, transfer line and oven was set at 90°C, 100°C and 80°C, respectively. The injection time was 0.5 min, the pressurizing time 4 min, and the column pressure was 30 psi.

The methods of sample preparation and analysis were modified from Suzuki *et al*. (1999). Frozen samples (ca. 100 mg) were ground to fine powder in liquid nitrogen and homogenized in 2 mL distilled water at 4^{o}C. The homogenate was collected in a 20-mL headspace vial and 1 mL of 2.5 µg mL^{-1} isopropanol was added as internal standard. The vials were immediately sealed by using PTFE-coated silicon rubber septa and aluminium crimp caps and incubated at 80^{o}C for 20 min. The system was calibrated with acetaldehyde (> 99.5%; Sigma-Aldrich) prior to the experiments.

Detection of superoxide (O_2^-) and hydrogen peroxide (H_2O_2) in leaves

Youngest fully-expanded leaves were sampled in the morning (1000 – 1130 h) in the glasshouse and transferred to a dim room adjacent to the glasshouse for detection of O_2^- and H_2O_2. Nitroblue tetrazolium (NBT; Loewe, Sauerlach) staining method of Doke and Ohashi (1988) was modified for detection of O_2^- in leaves. Small segments were cut from the leaves and quickly infiltrated with 0.1% (w/v) NBT in potassium phosphate buffer (50 mM, pH 6.4)

containing 10 mM NaN_3. Pressure was applied briefly and repeatedly until 80 – 90% of the area of leaf segments was infiltrated with the staining solution. Then, the leaf segments were incubated in the solution for 3 h in the dark under continuous agitation. Subsequently, the leaves were incubated in 100% ethanol at 50°C for 24 h in the dark to remove chlorophyll. The presence of O_2^- was visualized by dark-blue colour due to reduction of NBT to formazan.

The staining solution for H_2O_2 detection contained 0.1% (w/v) 3,3'-diaminobenzidine (DAB; Sigma-Aldrich) in MES buffer (10 mM, pH 6.5), as described by Thordal-Christensen *et al*. (1997). The procedures for infiltration and chlorophyll removal were the same as for the O_2^- detection. Brown precipitate resulting from DAB polymerization indicates the location of H_2O_2 formation in leaves.

Malondialdehyde (MDA) measurement

The extent of lipid peroxidation was estimated by measuring MDA concentration, a major product of lipid peroxidation. The method is described in Dhindsa and Matowe (1981). Frozen leaf and root samples (ca. 150 mg; see the section *Acetaldehyde measurement* for information about sampling) were ground in liquid nitrogen and 4.5 mL of 50 mM natrium phosphate buffer (pH 7.8) were added for extraction. The homogenate was centrifuged at 3000 g and 4°C for 15 min and 0.6 mL of the supernatant was mixed with 1 mL 0.6% (w/v) thiobarbituric acid in 20% (w/v) trichloroacetic acid (TCA). The samples were heated at 95°C for 20 min and then quickly cooled in an ice bath. After centrifugation at 3000 g for 15 min, absorption was measured at 532 nm and the absorption value at 600 nm was subtracted as the background. The MDA concentration was calculated by using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}.

Assay of antioxidant enzyme activities

The activity of SOD (EC 1.15.1.1) and CAT (EC 1.11.1.6) was analysed in the same leaf and root samples as used for the acetaldehyde and MDA analyses. The extraction method for the SOD assay was as described above for the MDA measurement. The supernatant after the first centrifugation step was used for the analysis. The SOD activity was determined by measuring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT), according to a method modified from Beyer and Fridovich (1987). The reaction mixture (3 mL) contained 50

mM natrium phosphate buffer (pH 7.8), 15 mM methionine, 0.73 mM NBT, 39 μM riboflavin and 100 μL of the sample extract. One unit of enzyme activity was defined as the amount of enzyme required for 50% inhibition of NBT reduction measured at 560 nm, compared with a blank containing 100 μL of 50 mM natrium phosphate buffer (pH 7.8) instead of the sample extract.

For the activity assay of CAT, frozen leaf and root samples (ca. 50 mg) were ground to fine powder in liquid nitrogen and homogenized in 2.5 mL of 200 mM natrium phosphate buffer (pH 7.8) containing 1% (w/v) polyvinylpolypyrrolidone. The homogenate was centrifuged at 3000 g and 4^{o}C for 15 min and the supernatant was taken for the analysis. The CAT activity was determined by monitoring the decrease in ultraviolet absorption of H_2O_2 at 240 nm as described by Azevedo *et al*. (1998). The reaction mixture (3 mL) contained 200 mM natrium phosphate buffer (pH 7.8), 100 mM H_2O_2 and 200 μL sample extract. Absorption changes were recorded in the samples every minute up to 4 min; thereafter the samples did not show significant changes in absorption. One unit of enzyme activity was defined as the amount of enzyme required to reduce the 240-nm absorbance by 0.1 in 1 min.

Reduced ascorbate (AsA) measurement

The AsA concentration was determined according to Law *et al*. (1983). The assay is based on the reduction of Fe^{3+} to Fe^{2+} by AsA in acidic solution. The Fe^{2+} forms a red chelate with bipyridyl absorbing at 525 nm. Frozen leaf and root tissues (ca. 100 mg; see the section *Acetaldehyde measurement* for information about sampling) were ground in liquid nitrogen and homogenized in 2 mL 6% (w/v) trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 3000 g and 4^{o}C for 15 min. The supernatant (200 μL) was mixed with 3 mL reaction solution containing 10% (w/v) TCA, 43% (w/v) H_3PO_4, 4% (w/v) bipyridyl in 70% (v/v) ethanol and 3% (w/v) $FeCl_3$. After incubation at 42^{o}C for 1 h, absorption was measured at 525 nm. The concentration of AsA was calculated by using a calibration with L-ascorbic acid.

RESULTS

Acetaldehyde accumulation

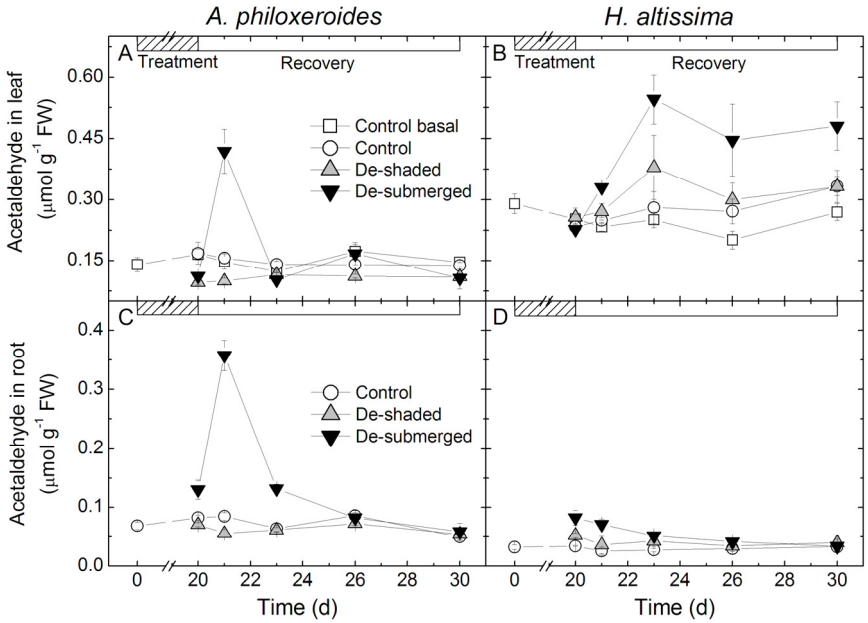

Fig. 1. Concentrations of acetaldehyde in leaves (A, B) and roots (C, D) of *A. philoxeroides* (A, C) and *H. altissima* (B, D) before (day 0) and at the end of the shading or submergence treatment (day 20) as well as during the subsequent 10-d recovery.

All plants were under the control condition until day 0. The shaded and submerged plants were brought back to the control condition on day 20. Plants staying under the growth condition (Control, white circle); plants covered with a shade cloth during day 0 – 20 (De-shaded, grey triangle); plants submerged during day 0 – 20 (De-submerged, black inverted triangle). Acetaldehyde concentration was analysed in ground tissues of youngest fully-expanded leaves and whole root systems. Also shown are data from basal leaves of control plants (Control basal, white square); these were the youngest fully-expanded leaves on day 0 and thus at the same age as the sample leaves of de-shaded and de-submerged plants. Whereas the youngest fully-expanded leaves were the same leaves throughout the experiment for de-shaded and de-submerged plants, which did not grow during the 20-d shading or submergence treatment, control plants continued to grow and form new leaves. Symbols are mean values (n = 4, ± s.e.).

The acetaldehyde concentration did not change substantially in leaves and roots of control or de-shaded plants for both *A. philoxeroides* and *H. altissima* throughout the experiment (Fig. 1). Under these conditions, leaves of *H. altissima* had about twice as much acetaldehyde as leaves of *A. philoxeroides* (Fig. 1A, B). After de-submergence on day 20, a transient but

significant increase in acetaldehyde was observed in leaves and roots of *A. philoxeroides* on day 21 (Fig. 1A, C). The values then returned to normal levels by day 23. In de-submerged plants of *H. altissima*, on the other hand, the acetaldehyde concentrations of leaves increased gradually in the first few days after de-submergence and stayed high until the end of the experiment, while roots exhibited only minor changes (Fig. 1D).

Formation of reactive oxygen species

Formation of O_2^- and H_2O_2 was detected by infiltration of leaf segments with NBT (Figs 2 and 3) or DAB (Figs 4 and 5), respectively. Control leaves of *A. philoxeroides* always showed some dark-blue staining after the NBT infiltration (Fig. 2; images of day 24 – day 30 are not shown), indicating the presence of O_2^-. The staining was sometimes confined to the regions around cut edges of the leaf segments but could be seen also in the middle part in other samples. After 20-d shading or submergence, the NBT infiltration did not give rise to the dark-blue colour in leaves of *A. philoxeroides* on day 20 (Fig. 2). The staining became recognizable in these plants on day 21, although still much weaker than in control plants. From day 23 onward, leaves of all treatments were similarly stained and could not be distinguished from each other. In marked contrast, leaf segments of *H. altissima* never showed dark-blue staining in any of the treatments, except in the very regions of cut edges (Fig. 3).

The DAB infiltration revealed formation of H_2O_2, manifested by brown colour, in leaves of *A. philoxeroides* shortly after de-submergence (Fig. 4). Unlike for O_2^- (Fig. 2), de-shaded plants resembled control plants with regard to H_2O_2, i.e. free of brown staining throughout the experiment (Fig. 4; images of day 24 – day 30 are not shown). The staining of de-submerged leaves disappeared on day 23, coinciding with the changes in acetaldehyde (Fig. 1A) and O_2^- (Fig. 2) in this species. Like seen for O_2^- (Fig. 3), leaves of *H. altissima* did not show any sign of H_2O_2 in all treatments (Fig. 5).

Lipid peroxidation

Lipid peroxidation was determined in leaves and roots by measuring the concentration of MDA. Leaf MDA concentrations were about three times higher in *H. altissima* than *A. philoxeroides* regardless of the treatments and leaf age (Fig. 6A, B). While the MDA levels increased only marginally from the youngest fully-expanded leaves to basal leaves in *A.*

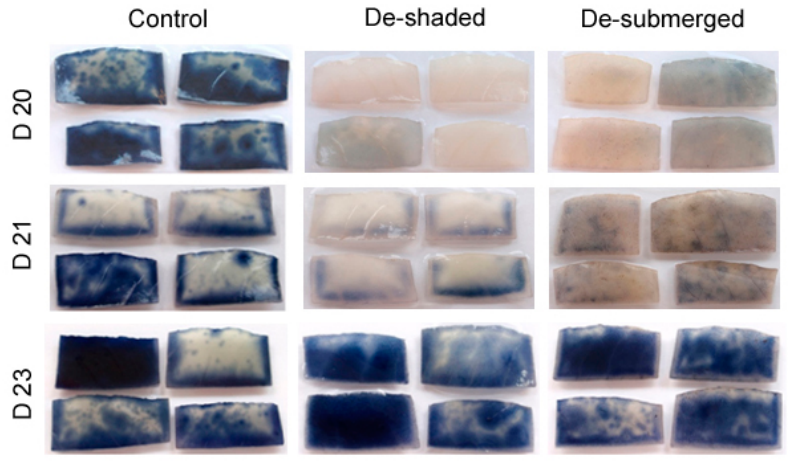

Fig. 2. Superoxide (O_2^-) detection in the youngest fully-expanded leaves of *A. philoxeroides* by nitroblue tetrazolium staining.

Images taken at the end of the shading or submergence treatment (day 20) and at the beginning of the recovery (day 21 and 23) are shown. Dark-blue colour of formazan indicates the location of O_2^- formation.

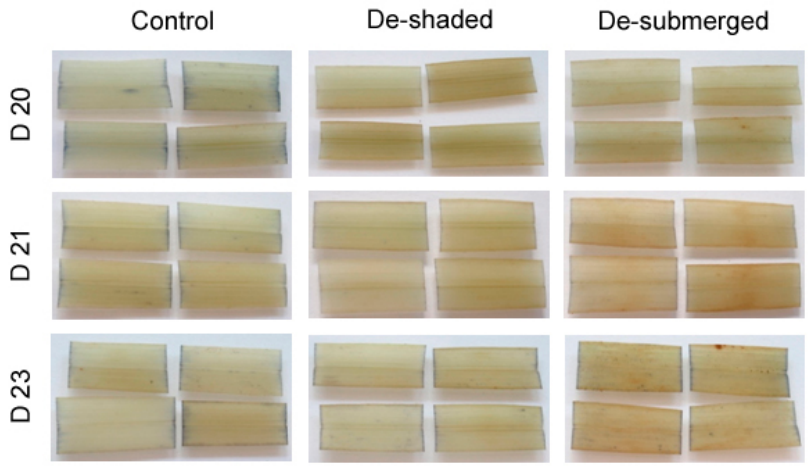

Fig. 3. Superoxide (O_2^-) detection in the youngest fully-expanded leaves of *H. altissima* by nitroblue tetrazolium staining.

Images taken at the end of the shading or submergence treatment (day 20) and at the beginning of the recovery (day 21 and 23) are shown. Dark-blue colour of formazan indicates the location of O_2^- formation.

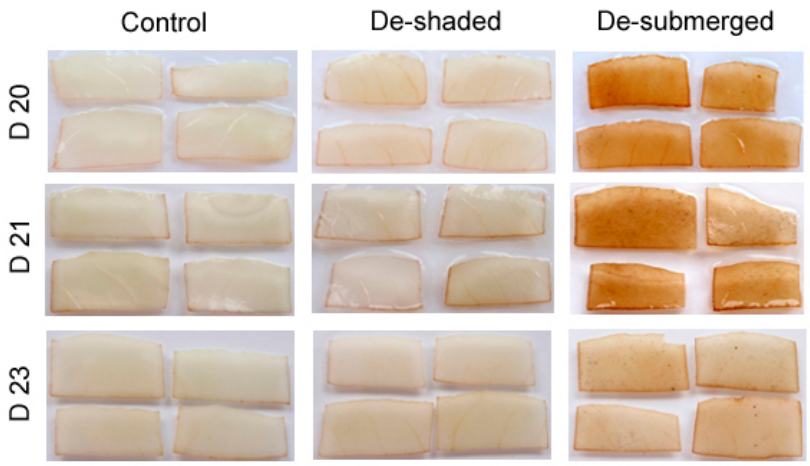

Fig. 4. Hydrogen peroxide (H_2O_2) detection in the youngest fully-expanded leaves of *A. philoxeroides* by 3,3´-diaminobenzidine staining.

Images taken at the end of the shading or submergence treatment (day 20) and at the beginning of the recovery (day 21 and 23) are shown. Brown colour indicates the location of H_2O_2 formation.

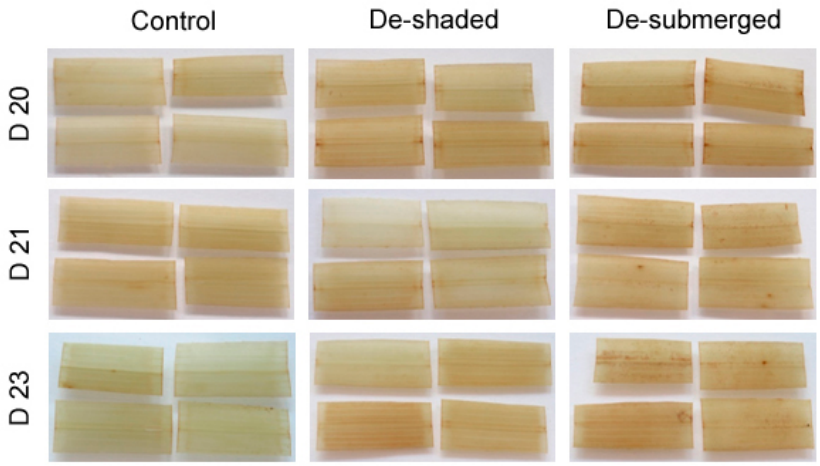

Fig. 5. Hydrogen peroxide (H_2O_2) detection in the youngest fully-expanded leaves of *H. altissima* by 3,3´-diaminobenzidine staining.

Images taken at the end of the shading or submergence treatment (day 20) and at the beginning of the recovery (day 21 and 23) are shown. Brown colour indicates the location of H_2O_2 formation.

philoxeroides under the control condition, basal leaves of *H. altissima* contained substantially more MDA than the youngest fully-expanded leaves. For both species, de-shaded plants and control plants contained similar amounts of MDA in the youngest fully-expanded leaves during the recovery period. In comoarison, de-submergence increased leaf MDA concentration from day 20 to day 21, which was more pronounced in *H. altissima* than *A. philoxeroides*. Subsequently, the MDA concentration stayed at this slightly increased level in de-submerged leaves of *A. philoxeroides* until day 26, whereas the values slowly but continuously declined in *H. altissima* after day 21 (Fig. 6A, B). The MDA concentrations of roots were similar in the two species and different treatments, except on day 20 for *A. philoxeroides* and day 23 for *H. altissima* when the values varied between the treatments (Fig. 6C, D).

Antioxidants

The antioxidant capacity was assessed by measuring SOD and CAT activities as well as AsA concentrations in leaves and roots of *A. philoxeroides* and *H. altissima*. Control plants of the two species had similar SOD activities in leaves (Fig. 7A, B). The SOD activity was reduced by 25 – 30% in de-submerged leaves of *A. philoxeroides* on day 20 and remained at this low level until day 23 (Fig. 7A). After 20 d of shading, leaves had the SOD activity similar to that of control plants, but the values tended to decline during the first few days after de-shading. Then, from day 23 to day 26, the SOD activity dramatically increased in leaves of de-submerged and de-shaded plants of *A. philoxeroides* and stayed high until day 30. In contrast, different treatments did not substantially affect the SOD activity in leaves of *H. altissima* (Fig. 7B). The values were highly variable from day to day in roots of *A. philoxeroides* (Fig. 7C); changes in de-submerged and de-shaded plants were not larger than those found in control plants. Roots of *H. altissima* showed different responses compared with leaves (Fig. 7D); the SOD activity in roots was significantly decreased for both de-submerged and de-shaded plants on day 20, which was followed by a gradual increase.

Changes in CAT activity largely paralleled the changes in the SOD activity in leaves of both species (Fig. 8A, B). However, a few differences between the CAT and SOD activities were found in *A. philoxeroides* (cf. Figs 7A and 8A). The CAT activity was decreased in leaves of both de-submerged and de-shaded plants of *A. philoxeroides* on day 20 and the recovery was observed from day 20 to day 23, i.e. before the recovery of the SOD activity.

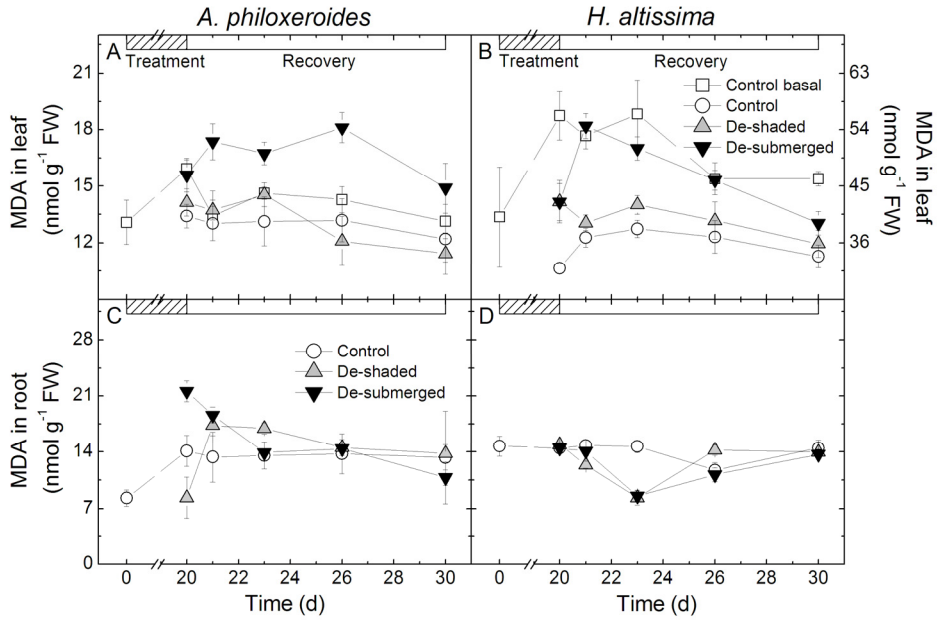

Fig. 6. Concentrations of malondialdehyde (MDA) in leaves (A, B) and roots (C, D) of *A. philoxeroides* (A, C) and *H. altissima* (B, D) before (day 0) and at the end of the shading or submergence treatment (day 20) as well as during the subsequent 10-d recovery.

For abbreviations of the samples, see legend to Fig. 1. Symbols are mean values (n = 4, ± s.e.).

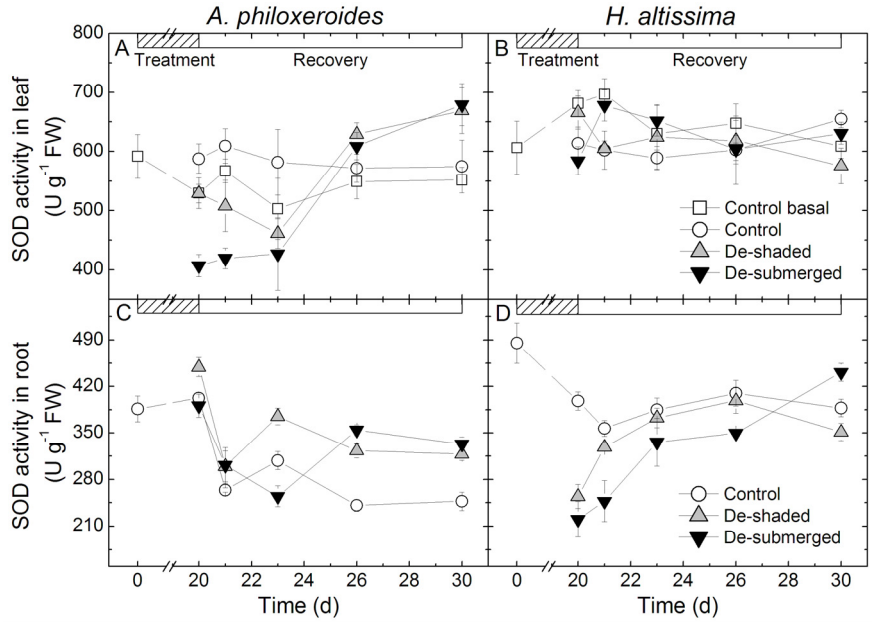

Fig. 7. Superoxide dismutase (SOD) activity in leaves (A, B) and roots (C, D) of *A. philoxeroides* (A, C) and *H. altissima* (B, D) before (day 0) and at the end of the shading or submergence treatment (day 20) as well as during the subsequent 10-d recovery.

For abbreviations of the samples, see legend to Fig. 1. Symbols are mean values (n = 4, ± s.e.).

Furthermore, basal leaves of this species had only 50% of the CAT activity in the youngest fully-expanded leaves. The CAT activity in roots was always low in *A. philoxeroides*, whereas control roots of *H. altissima* had twice the activity (Fig. 8C, D). Similar to SOD, the CAT activity was decreased in roots of de-submerged and de-shaded plants of *H. altissima* on day 20. The recovery started later for CAT than for SOD (cf. Figs 7D and 8D).

The youngest fully-expanded leaves contained more AsA than basal leaves in control plants of *A. philoxeroides* while the situation was the reverse for *H. altissima* (Fig. 9A, B). The leaf AsA concentration was strongly decreased in both species following de-submergence or de-shading. The levels recovered in all cases by day 23, with somewhat faster recovery in leaves of de-submerged plants of *H. altissima*. Generally, AsA concentration was much lower in roots than in leaves (Fig. 9C, D). Roots of *A. philoxeroides* always accumulated about twice as much AsA as roots of *H. altissima*. Changes in root AsA concentration were mostly small and only de-shaded plants of *A. philoxeroides* showed a significant increase between day 21 and day 23 (Fig. 9C), following the major recovery of leaf AsA concentration in these plants (Fig. 9A).

DISCUSSION

Upon return to aerobic conditions, ROS can lead to enhanced oxidation of cellular components (Møller *et al*., 2007) and an acetaldehyde "burst" (Monk *et al.*, 1987; Boamfa *et al*., 2005). Concomitant increase in O_2 concentration and light intensity exacerbates the situation in photosynthetic tissues, especially when plants are already suffering from lipid hydrolysation or peroxidation during submergence (Henzi and Braendle, 1993; Rawyler *et al*., 1999; Pavelic *et al*., 2000). Lipid peroxidation can be caused by increased ROS production and/or ineffective ROS detoxification (Drew, 1997; Pavelic *et al*., 2000). Negative correlations found between anoxia tolerance and lipid peroxidation in plants during submergence or after de-submergence (Blokhina *et al*., 1999; Santosa *et al*., 2007) underline the importance of protection against ROS for flood tolerance (Wollenweber-Ratzer and Crawford, 1994; Biemelt *et al.*, 1998). In *A. philoxeroides* and *H. altissima* showing, respectively, the escape and quiescence strategies of flood tolerance, we studied the changes in antioxidant and detoxification capacities during recovery after de-submergence.

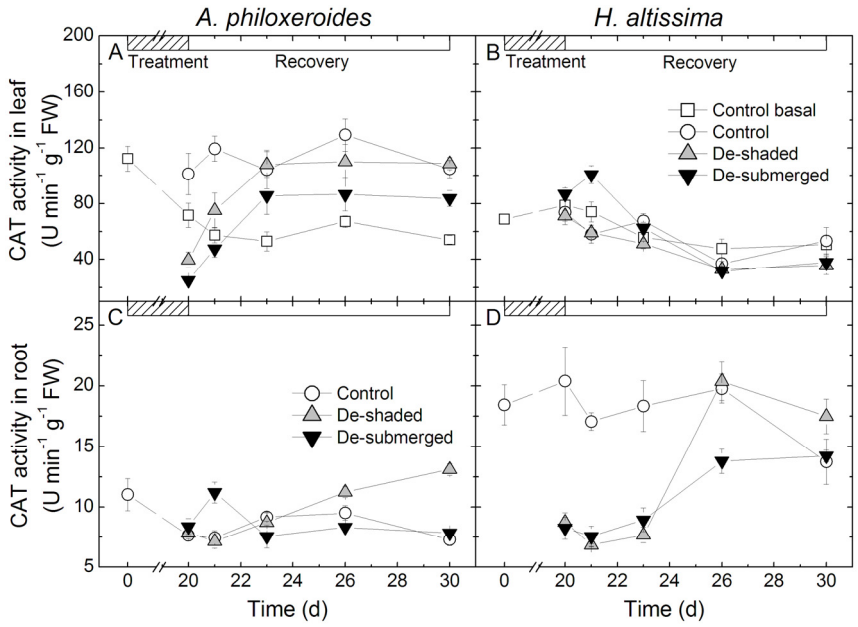

Fig. 8. Catalase (CAT) activity in leaves (A, B) and roots (C, D) of *A. philoxeroides* (A, C) and *H. altissima* (B, D) before (day 0) and at the end of the shading or submergence treatment (day 20) as well as during the subsequent 10-d recovery.

For abbreviations of the samples, see legend to Fig. 1. Symbols are mean values (n = 4, ± s.e.).

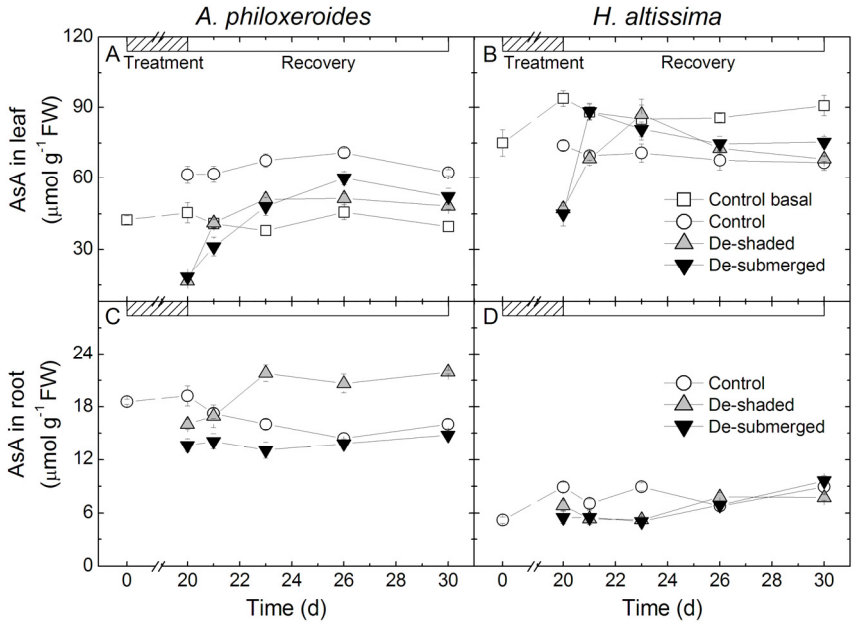

Fig. 9. Concentrations of reduced ascorbate (AsA) in leaves (A, B) and roots (C, D) of *A. philoxeroides* (A, C) and *H. altissima* (B, D) before (day 0) and at the end of the shading or submergence treatment (day 20) as well as during the subsequent 10-d recovery.

For abbreviations of the samples, see legend to Fig. 1. Symbols are mean values (n = 4, ± s.e.).

Acetaldehyde detoxification

Rapid increase in acetaldehyde has been reported for different species upon re-aeration after low-O_2 or anoxic treatments, e.g. in leaves of rice (Boamfa *et al*., 2005) or rhizomes of anoxia-intolerant *Glyceria maxima* (Monk *et al*., 1987). In the present study, little acetaldehyde was found in leaf and root tissues of *A. philoxeroides* and *H. altissima* at the end of submergence or shading (Fig. 1). Yet, de-submergence quickly increased acetaldehyde concentrations in both leaves and roots of *A. philoxeroides* from day 20 to day 21 (Fig. 1A, C). Low O_2 concentrations inside leaf and root tissues of submerged plants may have triggered fermentation and ethanol accumulation, resulting in rapid oxidation of ethanol to acetaldehyde, partly via CAT-mediated H_2O_2 scavenging, upon return to the atmospheric O_2 concentration. Subsequent decline of acetaldehyde in *A. philoxeroides* from day 21 to day 23 (Fig. 1A, C) suggests disappearance of ethanol and H_2O_2 (Fig. 4) and/or efficient enzymatic removal of acetaldehyde (Meguro *et al*., 2006; Mitsuya *et al*., 2009).

Slow and sustained accumulation of acetaldehyde found in leaves of *H. altissima* during the recovery period (Fig. 1B) is a symptom difficult to explain. Unlike *A. philoxeroides*, H_2O_2 was not detected in de-submerged leaves of *H. altissima* (Fig. 5) which retained relatively high antioxidant capacities during submergence (Figs 7B, 8B and 9B). Importantly, this much of acetaldehyde did not impede leaf growth recovery in de-submerged plants of *H. altissima*, which started between day 22 and day 23 under the same experimental conditions (Luo *et al*., 2010). Thus, it seems that acetaldehyde $\leq$ 0.5 μmol g^{-1} leaf FW is below toxic levels for *H. altissima*.

ROS and antioxidants

The dynamic adjustment and conservative behaviour found in growth and photosynthetic responses of *A. philoxeroides* and *H. altissima*, respectively, in the previous studies (Luo *et al*., 2009, 2010), were also evident in their antioxidative defense responses, especially in leaves. All three antioxidants examined, SOD, CAT and AsA, were downregulated in leaves of *A. philoxeroides* during submergence but recovered within 3 – 6 d after de-submergence (Figs 7A, 8A and 9A). Likewise, the levels of acetaldehyde, O_2^- and H_2O_2 were quickly normalized in a few days of recovery (Figs 1A, 2 and 4) and the increase in lipid peroxidation following de-submergence was very small in this species (Fig. 6A). In contrast, the activities

of SOD, CAT and AsA stayed relatively high (Figs 7B, 8B and 9B) and O_2^- and H_2O_2 could not be detected (Figs 3 and 5) in leaves of *H. altissima* under all conditions. This resembles the situation in other C_4 plants, maintaining high antioxidant capacities in leaves under stress conditions (e.g. Zhu *et al*., 2001; Casati *et al*., 2002; Stepien and Klobus, 2005). Although de-submergence induced a slow increase in acetaldehyde (Fig. 1B) and enhanced lipid peroxidation (Fig. 6B) in leaves of *H. altissima*, the latter change was reversed by the end of the experiment. It should be noted that leaves of *H. altissima* always contained higher amounts of both acetaldehyde and MDA than leaves of *A. philoxeroides*.

Unlike leaves, roots of *A. philoxeroides* showed no consistent variation in three antioxidants in response to the treatment (Figs 7C, 8C and 9C), whereas SOD and CAT activities were reduced in roots of *H. altissima* during submergence or shading (Figs 7D and 8D). The contrasting responses of antioxidants in leaves and roots of the two species probably reflect distinct metabolic activities and/or different ROS production and scavenging in these organs (Cavalcanti *et al*., 2007; Bian and Jiang, 2009; Skutnik and Rychter, 2009).

A peculiar observation made in leaves of *A. philoxeroides* was the detection of O_2^- (Fig. 2). In chloroplasts, electrons derived from water by photosystem II can be transferred to O_2 to form O_2^- at photosystem I (Asada, 1999). However, O_2^- is thought to be short-lived due to efficient scavenging by SOD (Asada, 1999), which is followed by removal of the dismutation product H_2O_2 by ascorbate peroxidase using two molecules of AsA. Dark-blue staining seen in control leaves of *A. philoxeroides* having both high SOD activities (Fig. 7A) and AsA concentrations (Fig. 9A) is therefore puzzling. Notably, emergence of O_2^- in de-submerged and de-shaded leaves of *A. philoxeroides* on day 23 (Fig. 2) concurred with full recovery of photosystem II activity measured in the corresponding leaves of this species under the same experimental conditions (Luo *et al*., 2010). The similar pictures found in de-submergence and de-shading plants also indicate a role of light in determining O_2^- formation in leaves of *A. philoxeroides*.

Concluding remarks

De-submergence responses of the detoxification systems differed between *A. philoxeroides* and *H. altissima*, especially in leaves: dynamic changes were observed in *A. philoxeroides* having the escape strategy as opposed to little or slow changes in *H. altissima* having the

quiescence strategy. Whereas the antioxidant capacities of these plants were often strongly influenced by light environments, toxic compounds (H_2O_2 in *A. philoxeroides*; acetaldehyde in both) and lipid peroxidation (leaves of both species) indicated detrimental effects of changing O_2 concentration which accompanies submergence and de-submergence.

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7. List of Tables and Figures

Introduction

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Luo *et al.*, 2010: Recovery dynamics of growth, photosynthesis and carbohydrate accumulation after de-submergence: A comparison between two wetland plants showing escape and quiescence strategies.

Luo *et al.*, 2010 submitted: How do antioxidative defense systems respond to de-submergence in wetland plants having escape and quiescence strategies?

8. List of abbreviations

ABA	abscisic acid
ADH	alcohol dehydrogenase
ALDH	acetaldehyde dehydrogenase
APX	ascorbate peroxidase
AsA	reduced ascorbate
ATP	adenosine triphosphate
CAT	catalase
DAB	3,3'-diaminobenzidine
DHA	dehydroascorbate
DHAR	DHA reductase
FID	flame ionization detector
Fm	maximal fluorescence intensity of PSII in the dark
Fm′	maximal fluorescence intensity of PSII in the light
Fo	minimal fluorescence intensity of PSII in the dark
Fs	steady-state fluorescence intensity of PSII in the light
ΔF/Fm′	effective quantum yield of PSII in the light
Fv/Fm	maximal quantum yield of PSII in the dark
GA	gibberellin
GSH	reduced glutathione
GSSG	oxidized glutathione
H_2O_2	hydrogen peroxide
MDA	malondialdehyde
MDHA	monodehydroascorbate
MDAR	MDHA reductase

NADP	nicotinamid adenine dinucleotide phosphate
NBT	nitroblue tetrazolium
NPQ	non-photochemical energy quenching in PS II
O_2^-	superoxide
$^1O_2$	singlet excited oxygen
OH^*	hydroxyl radical
OsBADH	betaine aldehyde dehydrogenase of rice
PAR	photosynthetically active radiation
PDC	pyruvate decarboxylase
PDH	pyruvate dehydrogenase
PEPC	phosphenolpyruvate carboxylase
PFK	phosphofructokinase
PK	pyruvate kinase
PSII	photosystem II
RGR	relative growth rate
ROS	reactive oxygen species
SK1	*SNORKEL1*, ethylene-responsive transcription factor
SK2	*SNORKEL2*, ethylene-responsive transcription factor
SOD	superoxide dismutase
SUB1A-1	*SUBMERGENCE1A-1*, ethylene-responsive transcription factor
tAPX	thylakoid-bound APX
TCA	trichloroacetic acid

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