Effects of flooding and sedimentation on the growth and physiology of two emergent macrophytes from Dongting Lake wetlands

Ying Pan [a,b,c], Yonghong Xie [a,b,*], Xinsheng Chen [a,b], Feng Li [a,b]

[a] Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Hunan, 410125, China
[b] Dongting Lake Station for Wetland Ecosystem Research, Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha 410125, China
[c] Graduate School of the Chinese Academy of Sciences, Beijing 100049, China

ABSTRACT

The aim of this study is to investigate the interaction between flooding and sedimentation on the performance of wetland macrophytes. Growth, biomass allocation, alcohol dehydrogenase (ADH) activity and non-structural carbohydrate content of the flooding-tolerant species *Carex brevicuspis* C.B Clarke and the flooding-intolerant species *Miscanthus sacchariflorus* (Maxim.) Hackel from the Dongting Lake wetlands were investigated experimentally using two water levels (0 and 40 cm, relative to the soil surface) and three burial depths (0, 5 and 10 cm). Biomass accumulation and relative growth rate (RGR) of both species were significantly inhibited by either higher water level or deeper burial, except for *C. brevicuspis* in the 5 cm burial depth + 0 cm water level treatment, suggesting that shallower burial would stimulate the growth of *C. brevicuspis*. Biomass allocation of both species was affected by water level alone, and more biomass was allocated to leaves at the 40 cm water level. ADH activity and soluble sugar content of *C. brevicuspis* were higher, but soluble sugar and starch contents of *M. sacchariflorus* were lower in the 40 cm than in the 0 cm water level treatments. Biomass accumulation, RGR and starch content of both species were unaffected by burial depth at the 40 cm water level. These results suggest that deeper burial only inhibits plant growth without flooding, and that both species can acclimate to flooding and/or sedimentation through adjustments of biomass allocation and carbohydrate metabolism.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Flooding and sedimentation are major stresses limiting plant growth and distribution in wetland ecosystems (Lowe et al., 2010), mainly through reducing oxygen availability in root zones (Little and Maun, 1996; Ferreira et al., 2009). Wetland plants can acclimate to anaerobic stress mainly through a series of morphological and physiological adjustments, such as shoot elongation, a higher shoot:root ratio, a shallower root system, as well as a higher alcohol dehydrogenase (ADH) activity and soluble sugar content (Little and Maun, 1996; Chen et al., 2005; Sun et al., 2010). Although many studies have been performed on the effect of flooding or sedimentation on plant growth, morphology and physiology, less attention has been paid to the interactive effects of the two stresses (Walls et al., 2005).

The interaction pattern between the two stresses is not consistent and may differ among species (Walls et al., 2005; Lowe et al., 2010). The processes of sediment transportation and deposition are strongly associated with flooding, indicating that flooding might play a more active role in regulating plant growth and distribution compared to sedimentation in wetland ecosystems (Pierce and King, 2007). A study by Lenssen et al. (2003) has found that both waterlogging and shade play important roles in inhibiting the growth of two waterlogging-tolerant species, while waterlogging has larger impact on the growth of waterlogging-intolerant species than does shade. Therefore, the interactions between sedimentation and flooding on plant growth might also be different due to the difference in flooding tolerance among species.

Dongting Lake, the second largest freshwater lake and the most typical river-connected lake in China, is usually flooded from May to October, when a large amount of sediment is deposited annually (Zheng et al., 2009). The purpose of this study was to investigate the interaction between flooding and sedimentation on the performance of wetland macrophytes. To this end, young ramets of two emergent macrophytes common to the Dongting Lake wetlands, the flooding-tolerant species *Carex brevicuspis* C.B Clarke and the flooding-intolerant species *Miscanthus sacchariflorus* (Maxim.) Hackel (Qin et al., 2010), were chosen to grow in two water levels and on three burial depths, and the morphology and physiology were investigated. *C. brevicuspis* usually occurs at the low elevation

and *M. sacchariflorus* at the high elevation. The following hypotheses were tested. Firstly, the growth will be inhibited by both higher water level and deeper burial for flooding-tolerant species *C. brevicuspis*, but plant growth is inhibited by higher water level alone for the flooding-intolerant species *M. sacchariflorus*. Secondly, both higher water level and deeper burial will lead to a higher shoot mass ratio, ADH activity and soluble sugar content, and a lower starch content for *C. brevicuspis*, and these responses will be observed in the environments of higher water level rather than deeper burial for *M. sacchariflorus*.

2. Materials and methods

2.1. Plant materials

Plant fragments of *C. brevicuspis* and *M. sacchariflorus* were excavated from stands in the Chunfeng Village (N29°13′49.72″, E113°02′32.79″), in the East Dongting Lake in March 2009, respectively. Plant fragments with roots were placed in separate plastic buckets, which contained 15 cm soil (containing 19 g kg^{-1} organic matter, 29 μg g^{-1} exchangeable N, and 19 μg g^{-1} exchangeable P), excavated from the community of *C. brevicuspis* in the Junshan, in the East Dongting Lake, to germinate new ramets. Organic matter, exchangeable P and exchangeable N were measured following the methods of Rayment and Higginson (1992), Stainton et al. (1977) and Bremner (1965), respectively. The plants were watered when necessary, and placed in a net-house, which was covered with one layer of nylon-net and the light was about 50% of full sun.

2.2. Experimental design

The experiment was started on 25 August 2009. A total of 30 ramets per species of similar size (5–8 leaves, about 20 cm in height) were transplanted individually in PVC tubes (20 cm in height, 11 cm in diameter, one ramet per tube). In each tube, 4 holes (1 cm in diameter) were evenly drilled at 6 cm above the tube bottom. Each tube was filled with 6 cm soil (the same soil used for plant incubation). Chen et al. (2011) reported that 9 cm burial significantly decreased the survival rate of *C. brevicuspis*, so 10 cm was chosen as the maximal depth. A two-way factorial design was applied in the experiment, which combined three burial depths (0, 5 and 10 cm) with two water levels (0 and 40 cm, relative to the soil surface). A total of six treatments were included in this experiment, each with five replicates. A total of 60 tubes were placed into five plastic buckets (88 cm × 67 cm × 63 cm, six treatments per species per bucket). The tubes were randomly placed in each bucket. One-time burial was conducted after transplantation for one week. The soil used for burial was the same as plant incubation. Water level was created by placing the tubes either in the bucket or on 40 cm high bricks. Water depth in each bucket was maintained at 46 cm depth. Tap water (containing 51.1 μg L^{-1} NH$_4^+$–N, 176 μg L^{-1} NO$_3^-$–N and 52.7 μg L^{-1} PO$_4^{3+}$–P, pH = 7.2) was supplied as needed and was completely replaced every two weeks.

2.3. Harvest

Plants were harvested on 11 November 2009. After harvest, the entire root system of each plant was carefully excavated and cleaned up by tap water, then brought to laboratory for measurement. Plants were divided into leaves, stems and roots (including rhizomes), and fresh mass was recorded (±0.0001 g). About half of the fresh roots were used for analysis of ADH activity. The remaining plant tissues were oven dried at 80 °C for 72 h, then reweighed to calculate a wet to dry conversion factor. Leaf mass ratio, stem mass ratio and root mass ratio were defined as the ratio of leaf mass, stem mass, root mass to biomass accumulation, respectively.

Biomass accumulation was the sum of leaf, stem and root masses. RGR was calculated using the following equations: RGR = (ln w_2 − ln w_1)/(t_2 − t_1), where w_1 was the initial dry mass, w_2 the dry mass at harvest time t_2, and (t_2 − t_1) the experimental time.

2.4. ADH activity

The root system was separated and 0.01–0.05 g of fresh root material was immediately kept in ice, then assayed (4 °C) in 5 ml assay mixtures (50 mM phosphate buffer pH 7.5, 5 mM MgCl$_2$, 1 mM phenylmethyl sulfonyl fluoride). Plant extracts were then centrifuged at 15,000 rpm for 20 min at 4 °C. Finally, the plant extracts were measured using diagnostic alcohol kits (Nanjing Jiancheng Bioengineering Institute, China). The results were expressed in enzyme units per milliliter of total serum protein; 1 U of the enzyme catalyzes the reduction of 1 nmol NAD+ per minute at a temperature of 37 °C.

2.5. Carbohydrate analyses

The content of non-structural carbohydrate (including starch and soluble sugar) was analyzed according to the methodology described by Yemn and Willis (1954). Dry root samples were ground to fine powder and extracted three times using 80% ethanol (v/v). The extract was then used for soluble sugar analysis after addition of anthrone reagent, followed by measurement of absorbance at 630 nm using a spectrophotometer. The residue remaining after soluble sugars extraction was dried and extracted using 30% perchloric acid and analyzed for starch (as glucose equivalent) using the anthrone reagent.

2.6. Statistical analysis

All statistical analyses were performed using SPSS13.0 package (SPSS Inc., USA). We tested the effects of water level and burial depth on biomass accumulation, RGR, biomass allocation, ADH activity, soluble sugar and starch contents by two-way ANOVA. Multiple comparisons of means were performed using Tukey's test at the 0.05 significance level and a Bonferroni correction for multiple comparisons was made when necessary. Dates were log$_{10}$-transformed if necessary to reduce heterogeneity of variances, and homogeneity was tested using Levene's test.

3. Results

3.1. Biomass accumulation and relative growth rate (RGR)

Both water level and burial depth significantly affected biomass accumulation of both species, with significant interactions ($P < 0.001$, Table 1). At the same burial depth, higher water level led to a lower biomass accumulation in *C. brevicuspis* ($P < 0.05$, Fig. 1). However, the effect of burial depth was dependent on water level. At the 0 cm water level, deeper burial led to lower biomass accumulation of both species, except for *C. brevicuspis* in the 5 cm burial treatment, which was 1.4 times higher than that in the 0 cm burial treatment (0.95 versus 0.70 g dry wt plant^{-1}, $P < 0.05$, Fig. 1). This result indicated that shallower burial would stimulate the growth of *C. brevicuspis* without flooding. At the 40 cm water level, biomass accumulation of both species was unaffected by burial depth ($P > 0.05$, Fig. 1). RGR showed the similar tendency as biomass accumulation, and was unaffected by burial depth at the 40 cm water level (Fig. 1). It was clear that deeper burial had negative effects on plant growth without flooding, but had no effect at higher water level.

Table 1
Summary of two-way ANOVAs for biomass accumulation, relative growth rate, biomass allocation and physiological characteristics of *Carex brevicuspis* and *Miscanthus saccharifloru* ramets growing on three sedimentation depths and in two water levels.

|	Species	n	Burial depth (B)		Water-level (W)		B × W	
			%SS	P-values	%SS	P-values	%SS	P-values
Biomass accumulation (g dry wt plant^{-1})	Carex	5	25%	0.000	59%	0.000	14%	0.000
	Miscanthus	5	23%	0.000	39%	0.000	20%	0.000
Relative growth rate (g g^{-1} dry wt day^{-1})	Carex	5	22%	0.000	67%	0.000	4%	0.042
	Miscanthus	5	16%	0.006	47%	0.000	11%	0.025
Root mass ratio (%)	Carex	5	4%	0.543	30%	0.003	2%	0.700
	Miscanthus	5	20%	0.073	4%	0.285	2%	0.762
Leaf mass ratio (%)	Carex	5	7%	0.178	49%	0.000	1%	0.709
	Miscanthus	5	3%	0.508	35%	0.001	7%	0.265
Stem mass ratio (%)	Miscanthus	5	11%	0.24	4%	0.323	4%	0.613
ADH (U ml^{-1} g^{-1} fresh wt)	Carex	4	18%	0.000	59%	0.000	18%	0.000
	Miscanthus	4	2%	0.299	15%	0.000	72%	0.000
Soluble sugar content (mg g^{-1})	Carex	4	43%	0.000	46%	0.000	2%	0.216
	Miscanthus	4	38%	0.000	35%	0.000	7%	0.053
Starch content (mg g^{-1})	Carex	4	12%	0.001	74%	0.000	4%	0.048
	Miscanthus	4	2%	0.409	83%	0.000	1%	0.597
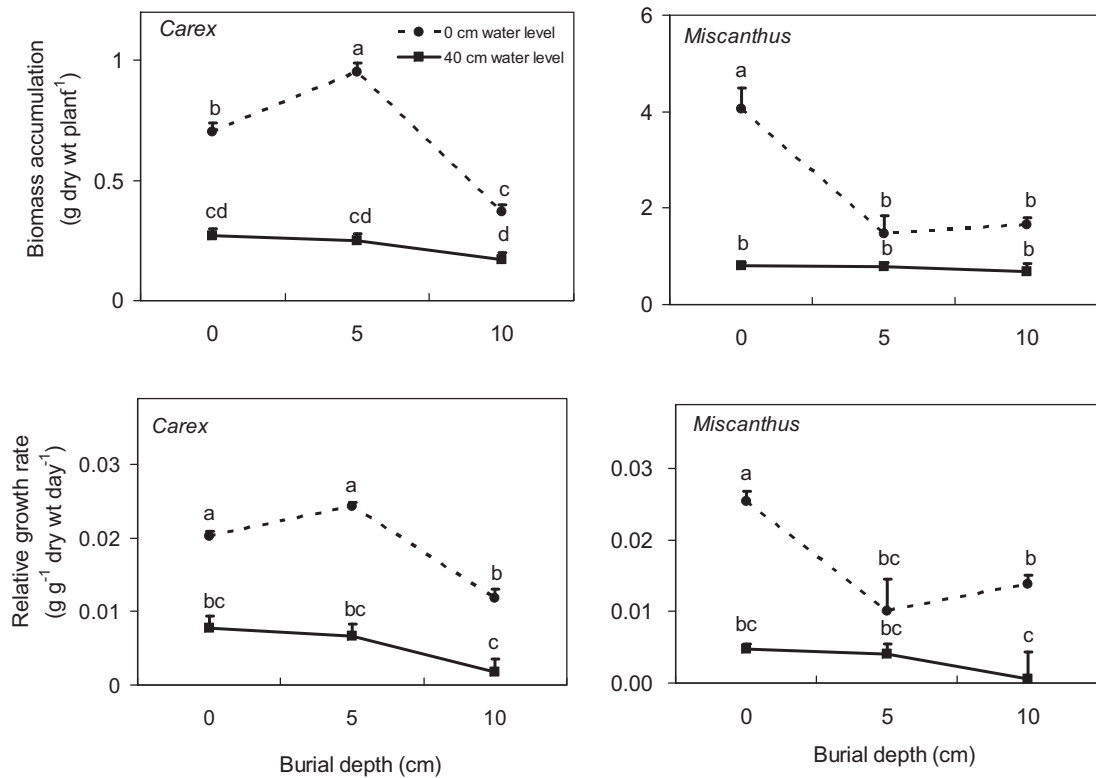
Fig. 1. Biomass accumulation and relative growth rate of *Carex brevicuspis* and *Miscanthus sacchariflorus* ramets growing on three sediment burial depths and in two water levels. Results are expressed as mean ± S.E. (*n* = 5). Different letters indicate significant difference among treatments at the 0.05 significance level.
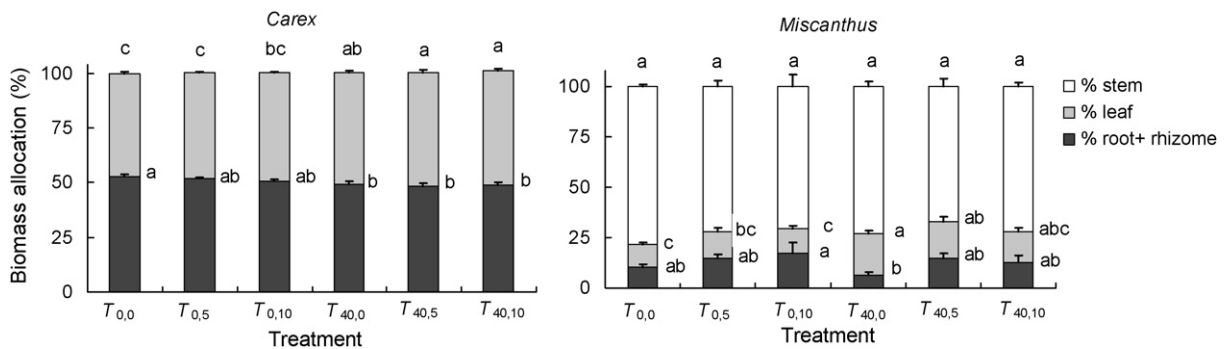

Fig. 2. Biomass allocation of *Carex brevicuspis* and *Miscanthus sacchariflorus* ramets growing on three sediment burial depths and in two water levels. Results are expressed as mean ± S.E. (*n* = 5). Different letters indicate significant difference among treatments at the 0.05 significance level.
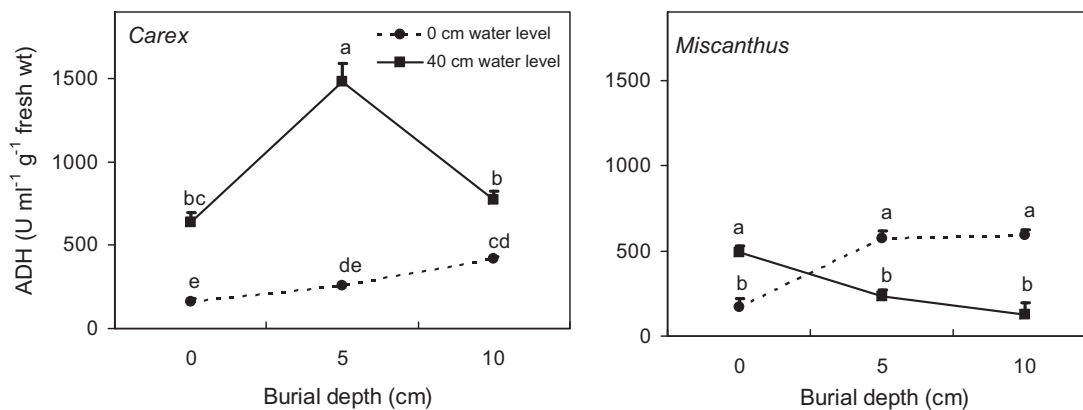
Fig. 3. ADH of *Carex brevicuspis* and *Miscanthus sacchariflorus* ramets growing on three sediment burial depths and in two water levels. Results are expressed as mean ± S.E. (n = 4). Different letters indicate significant difference among treatments at the 0.05 significance level.

3.2. Biomass allocation

Biomass allocation of both species was only affected by water level (P < 0.05, Table 1). For *C. brevicuspis*, higher water level led to a higher leaf mass ratio and a lower root mass ratio (P < 0.05, Fig. 2). For *M. sacchariflorus*, higher water level only led to a higher leaf mass ratio in the 0 cm burial treatment (P < 0.05, Fig. 2). These results indicated that water level rather than burial depth was the primary factor in regulating biomass allocation of both species.

3.3. ADH activity

ADH activity of both species was affected by water level and/or burial depth, with significant interaction (P < 0.001, Table 1). Higher water level led to a higher ADH activity in *C. brevicuspis*. However, higher water level led to a higher ADH activity in *M. sacchariflorus*

without burial, but a lower activity in the deeper burial treatments (P < 0.05, Fig. 3). Along with increasing burial depth, ADH activity increased in both species at the 0 cm water level, but decreased in *M. sacchariflorus* at the 40 cm water level (P < 0.05, Fig. 3). It was clear that the responses of ADH activity to the two stresses were species-specific, depending on stress type and intensity.

3.4. Soluble sugar and starch contents

Soluble sugar content of both species and starch content of *C. brevicuspis* was affected by both water level and burial depth, and starch content of *M. sacchariflorus* was only affected by water level (P < 0.01, Table 1). For *C. brevicuspis*, soluble sugar content increased with increasing water level and burial depth (P < 0.05, Fig. 4). Starch content decreased with increasing water level or increasing burial depth at the 0 cm water level, but had no change at the 40 cm water
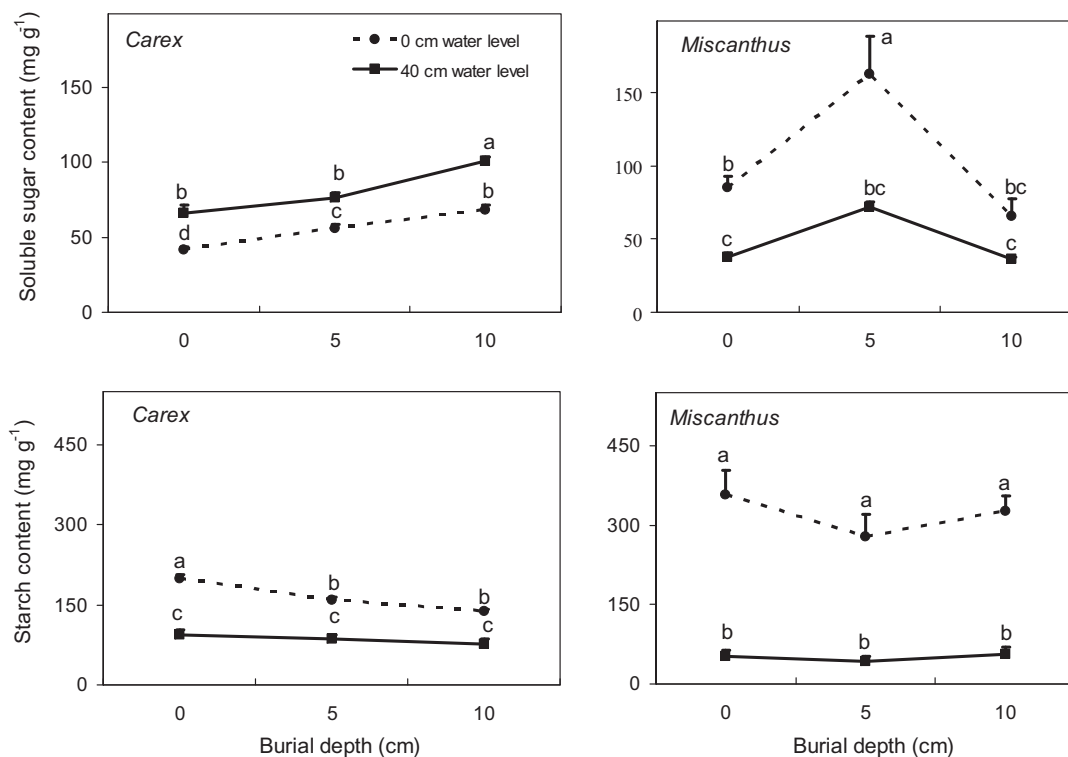

Fig. 4. Soluble sugar and starch contents of *Carex brevicuspis* and *Miscanthus sacchariflorus* ramets growing on three sediment burial depths and in two water levels. Results are expressed as mean ± S.E. (n = 4). Different letters indicate significant difference among treatments at the 0.05 significance level.

level. For *M. sacchariflorus*, higher water level led to a lower soluble sugar and starch contents, but the highest soluble sugar content was in the 5 cm burial depth + 0 cm water level treatment ($P < 0.05$, Fig. 4). Therefore, non-structural carbohydrate of *C. brevicuspis* was more sensitive to both flooding and sedimentation compared to *M. sacchariflorus*.

4. Discussion

Growth responses of both species were similar in this experiment. Either higher water level or deeper burial (10 cm) led to a lower biomass accumulation and RGR, but burial depth had insignificant effect on plant growth at the higher water level. This result supports our hypothesis 1 on the flooding-intolerant species *M. sacchariflorus*, rather than the flooding-tolerant species *C. brevicuspis*. Our results are also consistent with some studies, which have found that a dominance of flooding over sedimentation in regulating plant growth and distribution (Pierce and King, 2007). It seems that flooding is a stronger environmental stress on plant growth than does sedimentation in both species when the two stresses operate simultaneously. In contrast, Lowe et al. (2010) have found that sedimentation during flooding can further decrease the survival and growth of riparian plants. Therefore, the growth responses of wetland macrophytes to flooding and sedimentation might be species-specific.

The restraining effect on the growth of both species might be related to hypoxia in roots, since both flooding and sedimentation can limit plant growth through reducing oxygen availability in root zones (Little and Maun, 1996; Ferreira et al., 2009). ADH activity is usually considered as a direct indicator of oxygen deficiency in root tissues (Chen et al., 2005). A higher ADH activity in roots of both species might indicate that either higher water level or deeper burial reduces oxygen availability in root zones (Little and Maun, 1996; Ferreira et al., 2009). Compared to the single-factor response in *C. brevicuspis*, a higher ADH activity in the 5 cm burial depth + 40 cm water level treatment might indicate that both flooding and sedimentation consistently led to a higher level of oxygen shortage in roots. The acclimative strategy of both species to flooding and sedimentation can be associated with anoxia.

Our study also showed a dominance of water level over burial depth in regulating biomass allocation and non-structural carbohydrate content of both species. These results partly support our hypothesis 2 on *M. sacchariflorus*, rather than *C. brevicuspis*. Both species had similar responses to higher water level, such as a higher leaf mass ratio and a lower starch content. A higher leaf mass ratio is favorable for plants to emerge from water surface to acquire oxygen in the low-oxygen environments (Voesenek et al., 2004). A lower starch content might indicate consumption of a large amounts of carbohydrate due to fermentative metabolism (Chen et al., 2005), since starch can be transformed into soluble sugar to satisfy the energy requirement of necessarily physiological activity.

The responses of root mass ratio and soluble sugar content in both species were different under higher water level or deeper burial conditions. A lower root mass ratio in *C. brevicuspis* as a response to higher water level might be an effective strategy to reduce oxygen demand of below-ground tissues (Naidoo and Mundree, 1993). Besides, a higher soluble sugar content in *C. brevicuspis* as a response to higher water level and deeper burial might indicate that this plant can supply enough energy to maintain anaerobical respiration under anoxic conditions (Li et al., 2007). In contrast, deeper burial had no effect on soluble sugar content of *M. sacchariflorus* at the higher water level. The low-elevation species *C. brevicuspis* might be more sensitive to anoxia than does the high-elevation species *M. sacchariflorus*, as also suggested by Qin et al. (2010) and Luo et al. (2008). Therefore, both species can acclimate

to flooding or sedimentation through adjustments of biomass allocation and carbohydrate metabolism.

It was interesting that shallower burial stimulated the growth of *C. brevicuspis* rather than *M. sacchariflorus*, indicating that not all wetland macrophytes had the stimulative responsibility. Actually, the stimulated effects by shallow burial have been observed in plants in tidal wetland, salt marsh or dune ecosystems (Maun et al., 1996; Zhao et al., 2007; Sun et al., 2010). In order to restore degraded wetlands, using thin-layer sediment burial to promote the vigor of plants was effective in some species (Ford et al., 1999). The mechanisms may be related to the improved soil conditions for plant growth and temporary escape from harmful soil pathogens, which will stimulate root growth (Sun et al., 2010). It is clear that, many factors, such as stress intensity and biological differences among species, should be considered in order to restore degraded wetlands using shallower burial to promote plant growth.

References

Bremner, J.M., 1965. Inorganic forms of nitrogen. In: Black, C.A. (Ed.), Methods of Soil Analysis. American Society of Agronomy, pp. 1179–1237.

Chen, H.J., Qualls, R.G., Blank, R.R., 2005. Effect of soil flooding on photosynthesis carbohydrate partitioning and nutrient uptake in the invasive exotic *Lepidium latifolium*. Aquat. Bot. 82, 250–268.

Chen, X.S., Xie, Y.H., Deng, Z.M., Li, F., Hou, Z.Y., 2011. A change from phalanx to guerrilla growth form is an effective strategy to acclimate to sedimentation in a wetland sedge species *Carex brevicuspis* (Cyperaceae). Flora 206, 347–350.

Ferreira, C.S., Piedade, M.T.F., Franco, A.C., Gonçalves, J.F.C., Junk, W.J., 2009. Adaptive strategies to tolerate prolonged flooding in seedlings of floodplain and upland populations of *Himatanthus sucuuba*, a Central Amazon tree. Aquat. Bot. 90, 246–252.

Ford, M.A., Cahoon, D.D., Lynch, J.C., 1999. Restoring marsh elevation in a rapidly subsiding salt marsh by thin layer deposition of dredged material. Ecol. Eng. 12, 189–205.

Lenssen, J.P.M., Menting, F.B.J., van der Putten, W.H., 2003. Plant responses to simultaneous stress of waterlogging and shade: amplified or hierarchical effects? New Phytol. 157, 281–290.

Little, L.R., Maun, M.A., 1996. The 'Ammophila problem' revisited: a role for mycorrhizal fungi. J. Ecol. 84, 1–7.

Li, Y.Z., Xie, Y.H., Ren, B., Luo, W.B., Huang, J.S., 2007. Oxygen enhances the recovery of *Potamogeton maackianus* from prolonged exposure to very low irradiances. Aquat. Bot. 86, 295–299.

Lowe, B.J., Watts, R.J., Roberts, J., Robertson, A., 2010. The effect of experimental inundation and sediment deposition on the survival and growth of two herbaceous riverbank plant species. Plant Ecol. 209, 57–69.

Luo, W.B., Song, F.B., Xie, Y.H., 2008. Trade-off between tolerance to drought and tolerance to flooding in three wetland plants. Wetlands 28, 866–873.

Naidoo, N., Mundree, S.G., 1993. Relationship between morphological and physiological responses to waterlogging and salinity in *Sporobolus virginicus* (L.) Kunth. Oecologia 93, 360–366.

Maun, M.A., Elberling, H.D., Ulisse, A., 1996. The effects of burial by sand on survival and growth of Pitcher's thistle (*Cirsium pitcheri*) along Lake Huron. J. Coast. Conserv. 2, 3–12.

Pierce, A.R., King, S.L., 2007. The effects of flooding and sedimentation on seed germination of two bottomland hardwood tree species. Wetlands 27, 588–598.

Qin, X.Y., Xie, Y.H., Chen, X.S., 2010. Comparative study on the aerenchyma of four dominant wetland plants in Dong ting Lake. J. Wuhan Bot. Res. 28, 400–405 (in Chinese).

Rayment, G.E., Higginson, F.R., 1992. Australian Laboratory Handbook of Soil and Water Chemical Methods. Inkata Press, Melbourne.

Stainton, M.P., Capel, M.J., Armstrong, E.A.J., 1977. The Chemical Analysis of Freshwater. Canadian Fisheries and Marine Service Miscellaneous Special Publication.

Sun, Z.G., Mou, X.J., Lin, G.H., Wang, L.L., Song, H.L., Jiang, H.H., 2010. Effects of sediment burial disturbance on seedling survival and growth of *Suaeda salsa* in the tidal wetland of the Yellow River estuary. Plant Soil 337, 457–468.

Voesenek, L.A.C.J., Rijnders, J.H.G.M., Peeters, A.J.M., van de Steeg, H.M.V., de Kroon, H., 2004. Plant hormones regulate fast shoot elongation under water: from genes to communities. Ecology 85, 16–27.

Walls, R.L., Wardrop, D.H., Brooks, R.P., 2005. The impact of experimental sedimentation and flooding on the growth and germination of floodplain trees. Plant Ecol. 176, 203–213.

Yemn, E.W., Willis, A.J., 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57, 508–514.

Zhao, W.Z., Li, Q.Y., Fang, H.Y., 2007. Effects of sand burial disturbance on seedling growth of Nitraria sphaerocarpa. Plant Soil 295, 95–102.

Zheng, J.M., Wang, L.Y., Li, S.Y., Zhou, J.X., Sun, Q.X., 2009. Relationship between community type of wetland plants and site elevation on sandbars of the East Dongting Lake, China. Forest. Stud. China 11, 44–48.