Growth and physiological responses of saplings of two mangrove species to intertidal elevation

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ABSTRACT: Differences in growth and physiological characteristics of saplings of *Avicennia marina* and *Kandelia obovata* were compared among 3 intertidal elevations. Growth performance of *A. marina* generally followed the order of the middle > lower > upper elevation. At the middle elevation, *A. marina* had maximum values of tree height, branch number, crown length and diameter, shoot biomass and shoot to root biomass ratio (S:R). Differently, *K. obovata* exhibited best growth at the upper elevation, followed by the middle and lower elevation. Except for shoot biomass and S:R, all growth parameters of *K. obovata* showed a tendency to decline continuously with decreasing elevation. These different responses between the 2 species indicated that *A. marina* is more tolerant to low elevation than *K. obovata*. Decreasing elevation did not compromise gas exchange of *A. marina* leaves, while net photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO_2 concentration of *K. obovata* leaves declined sharply as the elevation decreased. Leaf chlorophyll (chl) *a:b* ratio and contents of chl *a*, total chl and total carotenoid showed no significant differences among elevations for both species. With decreasing elevation, activities of superoxide dismutase and peroxidase increased significantly for both species, while significant increase in malonaldehyde contents was only recorded for *K. obovata*. With decreasing elevation, proline accumulation in leaves was accelerated for *A. marina* but not for *K. obovata*. These physiological responses also confirmed that *A. marina* is more tolerant to low intertidal elevation than *K. obovata*.

KEY WORDS: Mangrove · *Avicennia marina* · *Kandelia obovata* · Sapling · Elevation · Growth · Physiology

INTRODUCTION

Distributed along tropical and subtropical coastlines, mangrove forests possess traits of both terrestrial and marine ecosystems. Mangrove forests are considered of great value in maintaining coastal biological diversity and providing economic products (Robertson & Duke 1987, Ye et al. 2005). In recent years, especially after the Indian Ocean tsunami on 26 December 2004, other ecological functions of mangrove forests have gradually received attention from all over the world, such as mitigating waves through hydraulic resistance (drag and impact force) owing to bottom roughness and vegetation, acceler-

ating sediment accumulation and purifying wastewater (Wong et al. 1997, Danielsen et al. 2005). However, mangroves around the world have for a long time been seriously threatened by sea level rise and human activities such as hydrologic modifications and excessive exploitation of coastal wetlands, which has made mangrove ecosystems vulnerable to both internal and external changes (Ellison & Farnsworth 1997, Allen et al. 2001, Krauss & Allen 2003, Ye et al. 2004, Prasad et al. 2011). Therefore, it is urgent to conserve and restore mangrove forests at both the national and global levels (Tamin et al. 2011). Most intertidal zones with high elevations in Xiamen, Fujian Province of China, were once occupied by

mangroves, but are now used for economical developments. Compensation measures for mangrove loss are usually done in low intertidal zones due to limited availability of mudflats (Lin et al. 2005). For successful mangrove reforestation it is therefore important to ascertain the effects of intertidal elevation on different mangrove species.

Intertidal elevation is one of the most important factors influencing mangrove growth and distribution (Lewis 2005), and to ascertain the existing hydrology (including depth, period and frequency of inundation) of natural extant mangrove ecosystems is crucial for successful mangrove rehabilitation. Komiyama et al. (1996) showed that survival and growth of seedlings of *Rhizophora apiculata* are significantly affected by an elevation difference of just 35 cm. Rather than responding passively to waterlogging, mangroves have evolved a series of morphological and physiological adaptations that allow avoidance or tolerance of low oxygen conditions induced by low elevation or waterlogging (Kozlowski et al. 1991). Youssef & Saenger (1996), for example, reported that for some viviparous mangrove species, roots conserve oxygen to maintain aerobic metabolism for longer periods during submergence. Roots and stems of mangroves also exhibit extensively developed aerenchyma to cope with waterlogging (Curran 1985).

However, elevation influences on mangrove species were mostly studied at seedling level (e.g. Smith 1987, Choy & Booth 1994, Ye et al. 2003, 2004, 2005), and only few studies have been conducted to determine how mangrove saplings adapt to elevation. In addition, previous results on response of mangroves to elevation or waterlogging mostly derived from greenhouse experiments (e.g. Pezeshki et al. 1990, Ellison & Farnsworth 1997, Delgado et al. 2001, Chen et al. 2004, Ye et al. 2004) in which wave action was absent. Actually, field studies on this topic are difficult because other factors, especially soil physico-chemical characteristics, vary significantly with intertidal elevation due to long-term tidal action, which may undermine the credibility and accuracy of mangroves' responses to elevation, e.g. when Kitaya et al. (2002) measured growth of 7 mangrove species at different elevations in the field. Therefore, field experiments are necessary to explore the exact effects of intertidal elevations on mangroves. We carried out a field experiment at several sites with different elevations but similar soil conditions, which would minimize the interference from edaphic heterogeneity.

Avicennia marina and *Kandelia obovata* are dominant species in Xiamen mangrove forests. They have pioneer properties and are considered suitable reforestation species. In mangrove forests, *A. marina* saplings can be found at all intertidal elevations (from landward margins to seawards), while *K. obovata* saplings mainly occur in mid to high tidal zones in spite of the fact that many seedlings can be found in low intertidal areas. So we hypothesize that *A. marina* saplings are more tolerant than *K. obovata* saplings to low intertidal elevation.

Therefore, we explored the responses of saplings of *Avicennia marina* and *Kandelia obovata* to elevation by comparing various growth and physiological parameters among different elevations of the same species. We also attempted to test the above hypothesis through studying the differences in responses to elevation between the 2 species, expecting to provide basic information for successful mangroves reforestation and their sustainable development.

MATERIALS AND METHODS

Experimental design

The study area is located along the coastline of Zengying (24° 33′ 9.24″ N, 118° 2′ 3.71″ E), Xiamen, China. According to the meteorological data of the recent 20 yr, Xiamen has an annual average rainfall of 1097 mm and annual average temperature of 20.8°C. The tides are typically semi-diurnal with an average amplitude of 4 m. The average seawater salinity is 28 ± 2 according to 12 monthly periodical measurements. The altitude of local mean sea level in Xiamen Bay was 365 cm above lowest sea level (Ruan et al. 2010). The intertidal zones of this area were once segmented into ponds separated by boulder strips for aquaculture (crab breeding). In recent years, these aquiculture ponds have been deserted and the cofferdams around them demolished, which ensured tidal exchange. To explore the suitable intertidal elevations for mangrove planting in these aquiculture zones, mechanically filling soils from nearby ponds were adopted at the beginning of 2004 to reconstruct study sites. Three adjacent ponds, with the same initial elevation (335 cm above the lowest sea level of Xiamen) and each with an area of ~1200 m², were transformed to 3 study sites of different elevations, namely, the lower, middle and upper elevation. Boulder strips were collected to enclose each of the 3 ponds, which separated the ponds from each other but did not affect tidal exchange. After that, surface soils were excavated from nearby ponds and transferred to 2 adjacent ponds. The 2 ponds

were then leveled off and compacted to reconstruct the middle elevation (the pond elevated by 50 cm after compaction) and the upper elevation (elevated by 100 cm after compaction). The unfilled pond, next to the middle elevation, was named the lower elevation. The 3 sites were parallel, each perpendicular to the coastline (Fig. 1a). All dredgerfill was collected from the same place, which had similar soil physicochemical properties to the unfilled pond (lower elevation). Prior to seedling plantation, the reconstructed sites were allowed to stabilize for several mo until the altitude of the elevations varied no more and soil physico-chemical properties became constant. The final steady elevation, measured on 28 May 2004, was 335, 375 and 415 cm above the lowest sea level of Xiamen for the lower, middle and upper elevations, respectively. Soil physico-chemical indexes (lower, middle and upper elevation; means ± SD), including pH (7.22 ± 0.12, 7.01 ± 0.43 and 7.30 ± 0.15, respectively), water content (42.38 ± 1.04, 41.74 ± 2.17 and 39.87 ± 4.32%), and soil salinity (28.31 ± 2.12, 29.03 ± 1.17 and 29.79 ± 1.98) were measured on 29 May 2004 and were not statistically different among the 3 elevations.

On 30 May 2004, the healthy mature propagules of *Kandelia obovata* collected from the mangrove forest in Yundang lagoon of Xiamen were directly planted at each elevation by two-thirds of the hypocotyl length inserted into the mud. On the same day, 21 mo old *Avicennia marina* seedlings of uniform size from a coastal mangrove nursery at Fenglin of Jimei, Xiamen, were transplanted at the 3 elevations. Each elevation was evenly divided into 8 plots that were perpendicular to the coastline and each with a width of 3 and length of 45 m. Seedlings of *A. marina* and propagules of *K. obovata* were planted in the plots next to each other at a row spacing of 0.5 × 0.5 m. The 2 plots at the edge of each elevation were excluded in order to avoid edge effects. Therefore, there were triplicates per elevation for each species (Fig. 1b). Mean inundation time, measured during one whole tide period of 15 d in August 2004, was ~10, 8 and 6 h d^{-1} (i.e. 5, 4 and 3 h per tide) for the lower, middle and upper elevations, respectively. The inundation time correspond with 42, 33 and 25% inundation, which fits the pattern of ~30% inundation time as described by Lewis (2005).

Growth parameter measurements

For each of the 3 replicate plots per elevation, 8 saplings of each species were randomly selected and tagged in October 2008 to measure tree height, crown length (calculated as the difference between the height of the tree and that of the first living branch), stem basal diameter, branch number and crown diameter. The tagged saplings were in the intermediate zone of each plot in order to avoid interspecific effects. At the end of the experiment, all tagged saplings were harvested. Individual harvested plants were separated into leaf, stem, and root components, and oven-dried at 80°C to determine the dry biomass of each component as well as shoot to root biomass ratio (S:R, shoot biomass divided by root biomass).

Physiological parameter analyses

For measurements of gas exchange, 8 mature and fully expanded leaves (2 sunny leaves at each of the 4 cardinal directions) of each tagged sapling were selected from each replicate in October 2008. The gas exchange measurements were conducted using a portable photosynthesis system (LI-6400XT) equipped with a 6.25 cm^2 leaf chamber. The following variables were evaluated: net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i). For each sampled leaf, the leaf chamber was clamped onto the leaf surface and tilted to maximize light incident on the leaf. Leaves are inclined at an angle of 0 to 45° from the horizontal (Tuffers et al. 1999). For
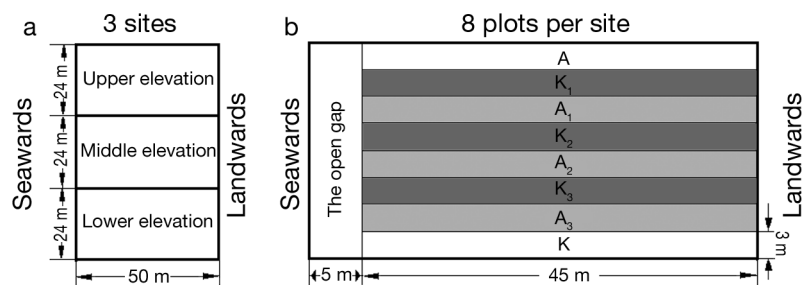

Fig. 1. *Avicennia marina* and *Kandelia obovata*. (a) Size and distribution of the 3 study sites. Bold lines: boundaries built of boulder strips. Altitude for the lower, middle and upper elevation is +335, +375 and +415 cm from lowest sea level datum of Xiaman, China, respectively. (b) Planting pattern of *A. marina* (A, triplicates: A_1, A_2 and A_3) and *K. obovata* (K, triplicates: K_1, K_2 and K_3) at each of the 3 elevations. A and K were discarded in consideration of edge effects. All seedlings were planted at a row spacing of 0.5 × 0.5 m. Each plot has 6 rows of plants, with 90 plants per row

each sampled leaf, 3 successive measurements were made at an interval of 25 s. All measurements were conducted on sunny days between 10 a.m. and 12 p.m. in the ambient CO_2 concentrations of 400 µmol mol^{-1}, photosynthetic photon flux density of 1600 µmol m^{-2} s^{-1} (the highest intensity of the day) and in the temperature range of 33 to 35°C. Water use efficiency (WUE) was calculated using the measured values of P_n and T_r (P_n/T_r). After these field photosynthesis measurements, the measured leaves were collected for laboratory chemical analyses.

Pigment contents were determined according to the methods described by Li (2000) with some modification. A tissue sample (~0.2 g) was cut from fresh mature leaves, weighed, and its area was measured with a CI-202 Leaf Area Meter (CID). Then, the midrib was excluded and the remaining leaf tissue was ground in a cold mortar with 10 ml of 80% acetone. The homogenate was centrifuged at 10000 g for 3 min and the absorbance of the supernatant was measured at 470, 646 and 663 nm. Then the concentrations of chlorophyll (chl) a, chl b, total chl and total carotenoid (Car) were determined according to the formulas given by Lichtenthaler & Wellburn (1983):

$$C_a = 12.21A_{663} - 2.81A_{646} ;$$
$$C_b = 20.13A_{646} - 5.03A_{663} ; \qquad (1)$$
$$C_{x+c} = (1000A_{470} - 3.27C_a - 104C_b)/229$$

where C_a, C_b and C_{x+c} are concentrations of chl a, chl b and total Car, respectively, while A_{663}, A_{646} and A_{470} are absorbance at 663, 646 and 470 nm. The content of pigment was expressed as pigment quantity per leaf area.

The methods for determination of leaf peroxidase (POD) activity, superoxide dismutase (SOD) activity and malonaldehyde (MDA) content in leaf were similar to those described by Li (2000) and further modified by Ye et al. (2005). Fresh leaf tissue (~0.3 g) was ground and homogenized with 1.5 ml phosphate buffer (pH 7.8, including 0.4% polyvinyl pyrrolidone) in an ice bath. The homogenate was centrifuged at 1500 g for 20 min and the supernatant was used to analyse the activities of POD and SOD as well as MDA content. For POD activity assay, 20 µl of the above extract was diluted to 1 ml and mixed with 3 ml reaction mixture (combined reagent from 500 ml of 0.1 mol l^{-1} pH 7.0 phosphate buffer, 280 µl analytically pure guaiacol and 190 µl analytically pure hydrogen peroxide). One unit of POD activity was indicated by an increase of 0.01 absorbance at 470 nm min^{-1} per gram of fresh weight. The SOD activity was measured by the degree of inhibition to

photo-reduction of nitro blue tetrazolium (NBT). Several reagents were mixed in a sequence of 2.4 ml of 62.5 mmol l^{-1} phosphate buffer, 0.2 ml of 0.06 mmol l^{-1} riboflavin, 0.2 ml of 30 mmol l^{-1} methionine, 0.1 ml of 0.003 mmol l^{-1} Na_2EDTA, 20 µl enzyme extract, and 0.2 ml of 1.125 mmol l^{-1} NBT. The enzyme solution was substituted by buffer solution to determine the maximum photo-reduction of NBT. The enzyme–substrate mixture was incubated at 400 lux for 25 min and the absorbance at 560 nm was measured. An enzyme unit was expressed as inhibition of maximum photo-reduction of NBT by 50%. For MDA content measurement, 3 ml of 0.5% thiobarbituric acid (TBA) was added into 2 ml enzyme extract and the reactivity was conducted in a boiling water bath for 10 min. Then the solution was centrifuged at 8000 g and 4°C for 5 min, and the absorbance of the supernatant at 532 and 600 nm was measured. The amount of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} (Heath & Packer 1968).

Determination of free proline was carried out following the method by Bates et al. (1973). Leaf tissue (~0.1 g) were homogenized in 10 ml 3% 5-sulfosalicylic acid solution. The homogenate was filtered through Whatman No. 1 filter paper. Then, 2 ml of the extract were added to 2 ml analytically pure glacial acetic acid and 2 ml analytically pure ninhydrin solution, and the mixture was incubated in a boiling water bath for 1 h. After cooling, 4 ml analytically pure toluene was added and strongly shaken. Then the absorbance at 520 nm was measured for final determination of free proline.

Data analysis

All values were expressed as mean ± SD of 3 replicates. The data for each variable were tested for normality and homogeneous variances, and no data transformation was needed. For each species, differences in growth and physiological response among 3 elevations (lower, middle and upper elevations) were analyzed by 1-way ANOVA. Differences among elevations and between species, and interactions of elevation and species were analyzed by 2-way ANOVA. Significant differences were at 0.05 levels and the p-value was adjusted by Bonferroni correction for multiple comparisons. If any significant difference was found, the Student-Newman-Keuls multiple comparison method was used to determine where the difference lay. All statistical analyses were performed using SPSS 16.0 for Windows.

RESULTS

Growth responses

Interactive effects of species and elevation were found very significant for tree height, stem basal diameter, branch number, crown length and diameter according to 2-way ANOVA (Table 1). Growth of *Avicennia marina* saplings was enhanced significantly by moderate water logging, with taller trees, higher branch number and larger crown diameter at the middle than the other 2 elevations (Fig. 2). The crown length differed little between the lower and middle elevations, but declined significantly at the upper elevation. However, in terms of stem basal diameter, *A. marina* had similar values irrespective of elevation. *Kandelia obovata* generally had significantly taller trees, larger stem basal diameter and higher branch number than *A. marina*, while no differences were found in crown length and diameter between the 2 species. Effects of elevation on growth of *K. obovata* was more pronounced than for *A. marina*, with significant differences occurring in all growth parameters among elevations (Table1, Fig. 2). Branch number and crown length of *K. obovata* showed a very obvious tendency to decrease with elevation, indicating strong inhibitory effects of prolonged waterlogging. However, the tree height, stem basal diameter and crown diameter showed no differences between the lower and middle elevations but a steep increase occurred at the upper elevation.

Growth responses, in terms of biomass of different plant parts and biomass partition to elevation were significantly different between the 2 species (Table 1, Fig. 3). *Kandelia obovata* had significantly more shoot and root biomass than *Avicennia marina*, while
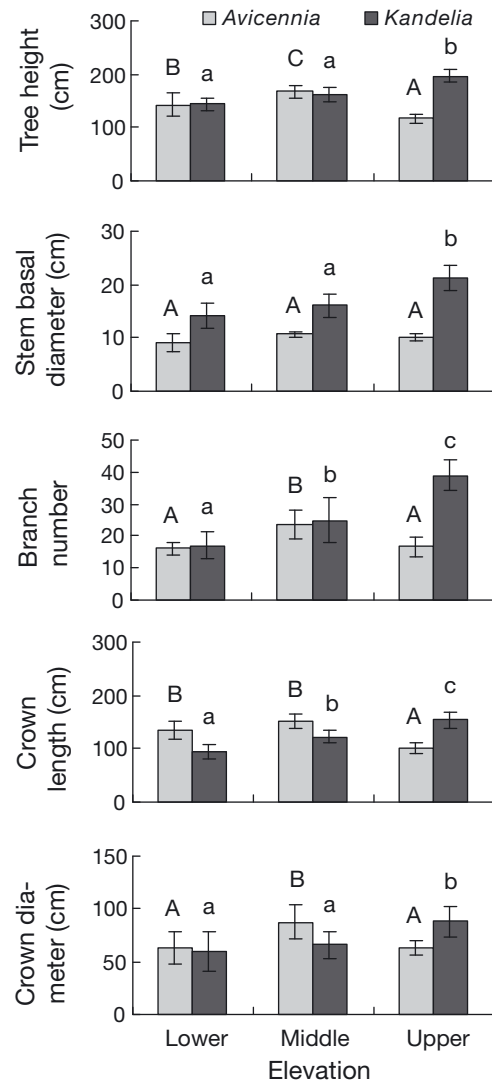

Fig. 2. *Avicennia marina* and *Kandelia obovata*. Tree height, stem basal diameter, branch number, crown length and diameter of saplings at 3 intertidal elevations. Data are mean ± SD of 3 replicates; different letters within species = significant difference at p < 0.05 according to 1-way ANOVA test

Table 1. *Avicennia marina* and *Kandelia obovata*. F-values of ANOVA results on tree height, stem basal diameter, branch number, crown length, crown diameter, shoot biomass (S), root biomass (R), and biomass ratio (S:R) of saplings. Significant effects: *0.01 < p < 0.05, **0.001 < p < 0.01, ***p < 0.001

Parameters	Sources of variation for 2-way ANOVA			1-way ANOVA: effects of elevation on respective species	
	Species (S)	Elevation (E)	S × E	*A. marina*	*K. obovata*
Tree height	40.345***	10.660***	48.564***	22.387***	41.844***
Stem basal diameter	181.621***	19.551***	13.505***	2.235	22.358***
Branch number	39.018***	25.616***	29.021***	7.484**	32.936***
Crown length	1.499	8.897***	45.326***	21.671***	34.083***
Crown diameter	0.005	4.721*	8.876***	7.054**	6.601**
Shoot biomass	502.669***	71.197***	65.101***	25.307***	87.131***
Root biomass	631.670***	133.629***	119.038***	1.033	146.027***
S:R	102.862***	74.603***	11.848***	46.610***	40.575***

tendency was reversed in the S:R ratio. Significant differences in shoot biomass of *A. marina* were found among elevations, with 12 and 30% higher values at the middle elevation than at the lower and upper elevations. However, *K. obovata* had the lowest shoot biomass at the lowest elevation, and no differences were found between the other 2 elevations. Root biomass of *A. marina* was constant regardless of elevation, while for *K. obovata*, decreasing elevation reduced root biomass by 67 and 29% for the lower and middle elevations, respectively. S:R ratio of *A. marina* was 12 and 35% higher at the middle elevation than at the lower and upper elevations. The decreasing elevation induced a sharp increase in S:R ratios for *K. obovata*, with the highest value occurring at the lower elevation, about twice the lowest value at the upper elevation, implying a shift in biomass partition from roots to shoots due to increasing waterlogging period.

Physiological responses

In general, prolonged waterlogging was more inhibitory for leaf gas exchange in *Kandelia obovata* than in *Avicennia marina* and all parameters excluding WUE were significantly lower in the former species than the latter (Table 2, Fig. 4). P_n, g_s, C_i and WUE in *A. marina* were unaffected by elevation. Although T_r declined significantly as the elevation decreased from the upper to the middle, no further
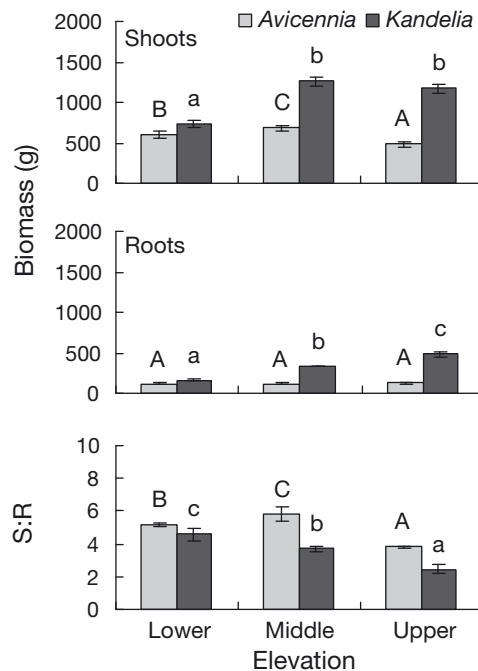

Fig. 3. *Avicennia marina* and *Kandelia obovata*. Shoot biomass (S), root biomass (R) and biomass ratio S:R of saplings at 3 intertidal elevations. Data are mean ± SD. See Fig. 2 for details

decrease in T_r was recorded as the elevation decreased from the middle to the lower. As for *K. obovata*, on the contrary, significant changes in P_n, T_r, C_i and g_s were consistent and all decreased considerably with decreasing elevation, suggesting that gas

Table 2. *Avicennia marina* and *Kandelia obovata*. *F*-values of ANOVA results on net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), water use efficiency (WUE), chlorophyll *a* (chl *a*), chl *b*, total chl, total carotenoid (Car), chl *a:b* ratio, superoxide dismutase (SOD) and peroxidase (POD) activities, and malonaldehyde (MDA) content of saplings. Significant effects: *$0.01 < p < 0.05$, **$0.001 < p < 0.01$, ***$p < 0.001$

| Parameters	Sources of variation for 2-way ANOVA			1-way ANOVA: effects of elevation on respective species	
	Species (S)	Elevation (E)	S × E	*A. marina*	*K. obovata*
P_n	249.886***	9.114***	6.528*	0.493	19.069***
T_r	339.556***	32.641***	5.167**	6.818**	46.471***
g_s	330.666***	13.508***	1.250	2.655	27.032***
C_i	101.019***	15.049***	8.243***	1.161	12.747***
WUE	20.301***	17.704***	13.821***	2.462	19.163***
chl *a*	75.661***	3.034	1.272	1.907	1.510
chl *b*	77.946***	8.575**	4.479*	0.971	6.572*
Total chl	89.869***.	4.523*	2.076	1.624	2.348
Total Car	53.157***	6.686**	1.519	1.786	3.885
chl *a:b*	0.556	2.407	0.986	0.393	1.528
SOD	49.976***	27.608***	0.207	10.503***	22.283***
POD	1.164	32.492***	2.520	28.248***	12.637***
MDA	91.499***	9.816***	3.574*	1.037	12.256***
Proline	120.632***	4.236*	2.818	3.905*	0.488
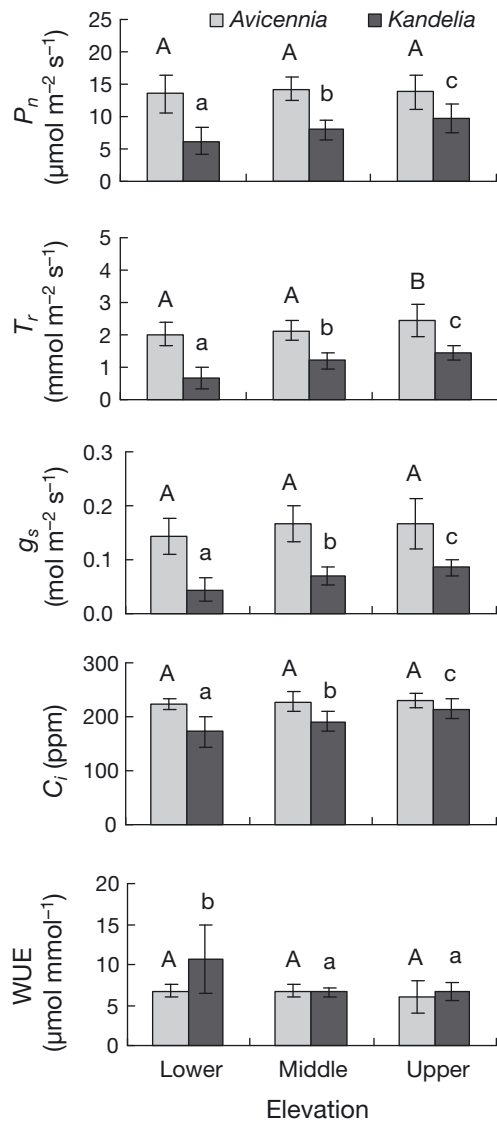
Fig. 4. *Avicennia marina* and *Kandelia obovata*. Net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and water use efficiency (WUE) of mature leaves of saplings at 3 intertidal elevations. Data are mean ± SD. See Fig. 2 for details

exchange was compromised by prolonged waterlogging. Unlike other parameters of gas exchange in *K. obovata*, WUE responded to prolonged waterlogging by a steep increase, as shown by the significantly higher value at lower elevation than at the other 2 elevations.

Pigment contents showed no interaction between elevation and species except for chl *b* (Table 2, Fig. 5). Pigment contents including chl *a*, chl *b*, total chl and total Car were significantly higher in *Kandelia obovata* than in *Avicennia marina*. For both species, chl *a*:*b* ratio and pigment contents excluding

chl *b* did not seem adversely affected by prolonged waterlogging according to one-way ANOVA. However, differences occurred when it came to the chl *b* content, which changed little in *A. marina*, but increased detectably in *K. obovata* as the elevation decreased from the upper to the middle.

SOD activity was greatly stimulated by decreasing elevation in both species (Table 2, Fig. 6). For *Kandelia obovata*, POD activity followed the same trend as that of SOD, with the values increasing steeply as the elevation decreased from the upper through the middle to the lower. However, in *Avicennia marina*, significant increase in POD activity did not occur until the elevation decreased to the lowest. MDA content was less variable and much lower in *A. ma-*
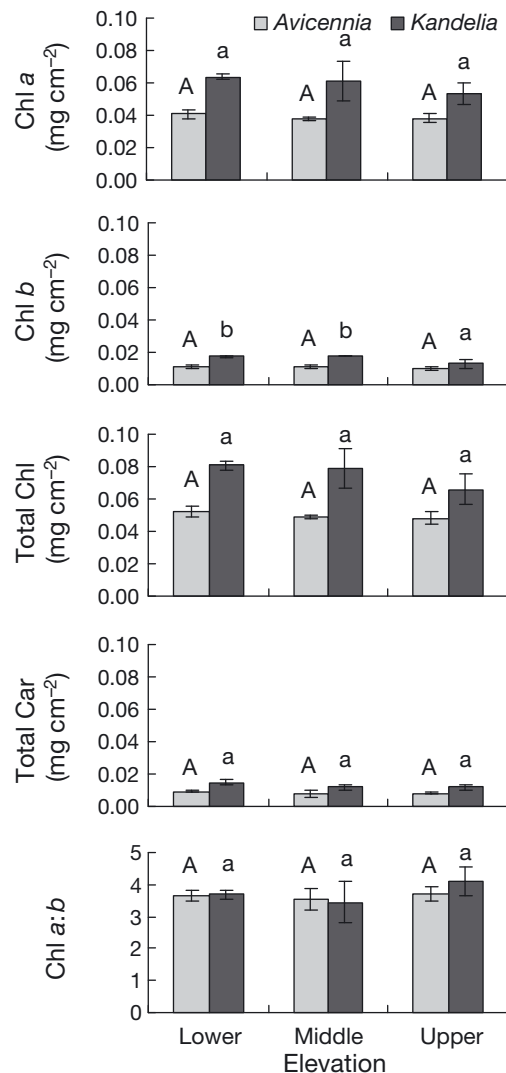

Fig. 5. *Avicennia marina* and *Kandelia obovata*. Contents of chl *a*, chl *b*, total chl, total Car, and chl *a*:*b* ratio in mature leaves of saplings at 3 intertidal elevations. Data are mean ± SD. See Fig. 2 for details
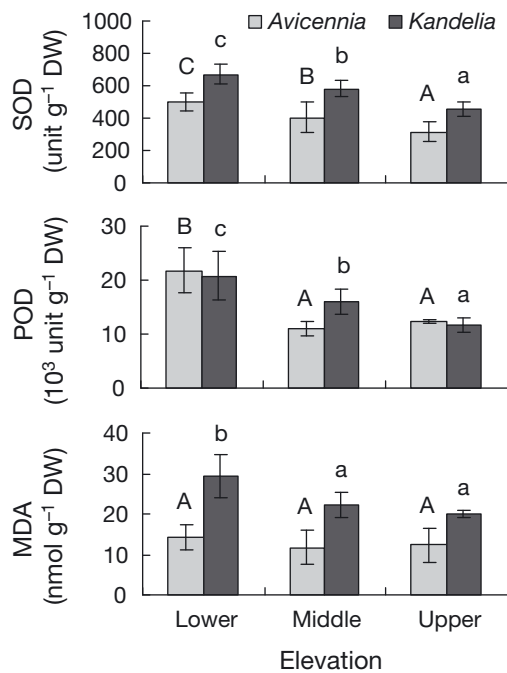
Fig. 6. *Avicennia marina* and *Kandelia obovata*. Activities of superoxide dismutase (SOD) and peroxidase (POD) and malonaldehyde (MDA) content in mature leaves of saplings at 3 intertidal elevations. Data are mean ± SD. Units per g dry weight (DW). See Fig. 2 for details

rina than in *K. obovata*. MDA content did not change with elevation in *A. marina* but increased significantly with decreasing elevation in *K. obovata*, and the value in *A. marina* was ~49, 53 and 62% that in *K. obovata* for the lower, middle and upper elevations, respectively.

The contents of free proline increased significantly with decreasing elevation in *Avicennia marina*, with the highest value found at lower elevation, ~12 and 49% higher than the middle and upper elevations, respectively (Table 2, Fig. 7). The content in *Kandelia obovata* varied little with elevation and was only 29, 28 and 37% of that in *A. marina* for the lower, middle and upper elevations, respectively.
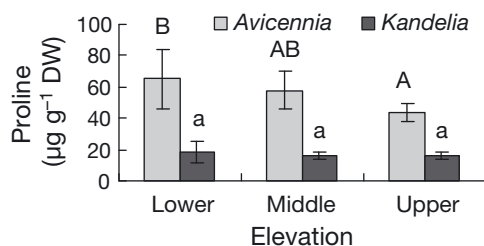

Fig. 7. *Avicennia marina* and *Kandelia obovata*. Proline content in mature leaves of saplings at 3 intertidal elevations in µg per g dry weight (DW). Data are mean ± SD. See Fig. 2 for details

DISCUSSION

Growth responses

Prolonged waterlogging restrains the development of mangroves, expressed as small leaf area and leaf number (Pezeshki et al. 1990), causing fewer branches, shorter stems and smaller biomass, as well as altered crown dimensions (Ellison & Farnsworth 1997, Chen et al. 2004). In our study, growths of both *Avicennia marina* and *Kandelia obovata* saplings were significantly affected by decreasing elevation, while their responses to elevation were different.

Saplings of *Avicennia marina* exhibited best growth performance at the middle elevation (waterlogged for 8 h d^{-1}), with the tallest trees, highest branch number, largest stem basal, as well as largest crown dimensions. The crown dimensions, involving the crown length and crown diameter, has important consequences for the performance of trees, as it determines total leaf area and foliage distribution, and hence, light capture and carbon gain (Kohyama 1987, King et al. 2006, Poorter et al. 2012).

Unlike early seedlings, *Avicennia marina* saplings have a well developed aerenchyma that accounts for ~70% of the root volume (Curran 1985). Accordingly, root ventilation is efficient under prolonged waterlogging and consequently conferred a strong ability to avoid or resist damage induced by prolonged waterlogging (Naidoo et al. 1997). With the decrease in elevation from upper to middle, water availability increased, accompanied by the efficient root ventilation, leading to significantly strengthened growth. However, the further decrease in elevation inevitably compromised the growth of *A. marina* saplings, as indicated by the significantly decreased tree height, branch number and crown diameter at the lower elevation compared to the middle elevation. Hovenden et al. (1995) reported that aerenchyma in 1 yr old *A. marina* seedlings with pneumatophores can fulfill the oxygen requirements of the root system when waterlogged <3.5 h per tide (~7 h d^{-1}). In our study, the *A. marina* saplings seemed to tolerate waterlogging well for up to 8 h (middle elevation), but when the roots were isolated from the air for a longer time (10 h, lower elevation), the oxygen storage within the aerenchyma could eventually be depleted and lead to the decline of various growth parameters (Youssef & Saenger 1996). In addition, we clearly showed that growth differences for *A. marina* between the middle and lower elevations were generally smaller than those between the middle and upper elevations, similar to that observation by Ellison & Farnsworth

(1997) for 2.5 yr old *Rhizophora mangle*. This phenomenon seemingly implied that the limited tidal action at the upper elevation was strongly inhibitory for growth of *A. marina* saplings.

The influence of waterlogging was more profound on saplings of *Kandelia obovata*, as shown by greater differences in growth among elevations (Table 1, Fig. 2). Furthermore, growth responses of *K. obovata* to elevation followed a quite different pattern, with the best growth performance occurring at the upper elevation (waterlogged for 6 h d^{-1}), indicating that increasing waterlogging had adverse effects on growth of *K. obovata* saplings. These results matched the distribution of *K. obovata* saplings in mid to high intertidal areas in the field (Fan 2000). Furthermore, field observation has demonstrated increased leaf senescence of *K. obovata* saplings at the lower elevation.

Interactive effects of elevations and species were very significant in all growth parameters (Table 1). Previous studies on mangroves showed that the more stress-tolerant species had lower relative growth rates (RGRs), biomass and leaf area than less stress-tolerant species (Ball & Pidsley 1995, Ball et al. 1997, Ball 2002). Mckee (1995) reported that mangrove species with intrinsically slow growth rates can maximize tolerance under stressful environments. Compared to *Kandelia obovata*, *Avicennia marina* generally had significantly shorter and thinner stems, fewer branches and less biomass, so it was more tolerant to varying elevations. It appears that increasing waterlogging-tolerance of *A. marina* is at the expense of growth potential, similar to conclusions from other studies comparing waterlogging-tolerance of 2 closely related mangroves species such as *Bruguiera gymnorrhiza* and *K. obovata* (Ye et al. 2003), and *A. marina* and *Rhizophora stylosa* (He et al. 2007).

The relatively lower root biomass of *Kandelia obovata* under prolonged waterlogging decreases the oxygen demand by root tissue, shortens the oxygen diffusion path to the growing root tips and decreases the requirement of oxygen for external rhizosphere oxidation (Ye et al. 2004), which mitigates root zone hypoxia and consequently enhance plants′ resistance to prolonged waterlogging (McKee 1996). In other words, the biomass shift from roots to shoots can be considered an adaptive mechanism by mangrove plants to cope with waterlogging. Seedlings of *K. obovata* in simulated semidiurnal tide increased shoot to root biomass ratio when subjected to prolonged waterlogging (Ye et al. 2003).

Avicennia marina saplings also increased S:R ratio as elevation decreased from the upper to the middle, indicating a similar strategy to resist waterlogging.

However, different from *Kandelia obovata*, *A. marina* increased biomass investment to roots with a further decrease in elevation, resulting in decreased S:R ratio from the middle to the lower elevation. The growth performance of *A. marina* saplings at the lower elevation indicated that other strategies might be involved. Prolonged waterlogging can increase the parenchymatous tissue in some plants (Armstrong 1979, Kozlowski 1997) and accelerates the formation of aerenchyma within *A. marina* seedlings (Xiao et al. 2009). Thus, with increasing waterlogging, *A. marina* saplings allocate relatively more energy for root development, which permits sufficient oxygen supply for aerobic respiration through pneumatophores.

Physiological responses

Waterlogging is reported to directly induce stomatal closure (Ashraf 2003), which consequently leads to decreasing g_s and C_i. A positive relationship is found between g_s and P_n, and the former variables are also tightly associated with T_r (Naidoo 1983, McLeod et al. 1999, Ashraf 2003). Species differing in tolerance to waterlogging may show different responses with respect to their gas exchange. Chen et al. (2005) reported that decline in P_n, T_r, g_s and C_i in *Kandelia obovata* seedlings occurs under prolonged waterlogging, while WUE is lower during medium periods and higher during long period of inundation. Similar findings were reported for *Bruguiera gymnorrhiza* (Naidoo 1983, Youssef & Saenger 1998) and *Rhizophora mangle* (Ellison & Farnsworth 1997). However, gas exchange of mature *Avicennia marina* trees was not adversely affected even when all pneumatophores were completely submerged by seawater for up to 5 d (Naidoo et al. 1997). In our study, the parameters of gas exchange, P_n, T_r, g_s and C_i almost changed uniformly with elevation for both *A. marina* and *K. obovata*, indicating strong relationship among these parameters. However, the change differed significantly between species, implying a different tolerance to waterlogging. Except for T_r at the upper elevation, gas exchange by *A. marina* was not compromised by prolonged waterlogging, as shown by the unchanged P_n, g_s, C_i and WUE, indicating that *A. marina* saplings are well adapted to prolonged waterlogging. A similar conclusion was also reached by Naidoo et al. (1997). The response in gas exchange by *K. obovata* was distinct from that of *A. marina*, but somewhat in accord with its growth performance. The decline in P_n and T_r was closely

accompanied by a decrease in g_s and C_i under prolonged waterlogging, which led us to conclude that prolonged waterlogging depresses P_n and T_r mainly by stimulating stomatal closure and reducing intercellular CO_2 concentration. However, it is interesting that WUE in *K. obovata* saplings was significantly higher at the lower elevation than the other 2 elevations. A similar phenomenon was observed for *K. obovata* seedlings (Chen et al. 2005).

Previous studies on seedlings of *Kandelia obovata*, *Bruguiera gymnorrhiza* and *Rhizophora mangle*, demonstrated that prolonged waterlogging can induce significant variation in pigment contents as well as in the ratio of chl *a* to chl *b* (Ellison & Farnsworth 1997, Ye et al. 2004). However, in our study, chl *a:b* ratios and pigment contents were generally insusceptible to prolonged waterlogging for both *Avicennia marina* and *K. obovata*. This contradiction between the current and previous studies may be ascribed to the different growth stages (saplings versus seedlings). Actually, when chronically exposed to prolonged waterlogging, mangroves can gradually adjust their physiology and morphological structure to the stressing conditions (Ball & Pidsley 1995, Youssef & Saenger 1996). In addition, change in pigment contents is considered one of the reasons for variation in P_n. For *A. marina* saplings, the constant responses in pigment contents and chl *a:b* ratios among elevations was in accord with its responses in gas exchange. Although pigment contents in *K. obovata* saplings were not affected and chl *b* even stimulated by prolonged waterlogging, P_n declined sharply from the upper to the lower elevation. A similar phenomenon has also been recorded in *K. obovata* seedlings (Ye et al. 2003) and some herbaceous species (Ashraf 2003). The asymmetrical relationship between the pigment contents and P_n in *K. obovata* confirmed our previous conclusion that stomatal factors were the main reason for the decreased P_n, and the increase in chl *b* content in *K. obovata* compensated for the P_n loss, which was induced by stomatal limitation.

Lipid peroxidation is an indicator of oxidative damage that can be induced by waterlogging (Bowler et al. 1992, Ye et al. 2003) or other environmental stressors (Takemura et al. 2000). Under prolonged waterlogging, an immediate increase in activities of SOD and POD was found in seedlings of *Kandelia obovata* and *Bruguiera gymnorrhiza* (Ye et al. 2003). MDA is a major product of lipid peroxidation and change in its contents reflects the level of injury to plants under stress. Significant increase in MDA content was recorded by Chen et al. (2005) in *K. obovata* seed-

lings under prolonged waterlogging. In our study, saplings of both *Avicennia marina* and *K. obovata* responded to prolonged waterlogging by increasing the activities of SOD and POD, suggesting that both species have some degree of resistance to waterlogging. The MDA contents showed a tendency to increase with decreasing elevation in *K. obovata* but remained constant regardless of elevation in *A. marina*, indicating that lipid peroxidation was activated by prolonged waterlogging in *K. obovata* but not in *A. marina*. In addition, the MDA contents in *K. obovata* were significantly higher than in *A. marina* at all elevations, which implied that lipid peroxidation was more active in *K. obovata*.

Free proline accumulates in response to a wide range of environmental stressors in many plants (Dopp et al. 1985, Delauney & Verma 1993). In addition to its conventional role as a compatible solute to counter osmotic challenge (Delauney & Verma 1993), proline accumulation can also confer adaptive advantages by its ability to detoxify free radicals through formation of long-lived adducts (Girousse et al. 1996, Hare & Cress 1997) and to store energy for recovery from stress (Atkinson 1997, Hare & Cress 1997). This makes proline accumulation a great stress reliever. In our study, saplings of *Avicennia marina* and *Kandelia obovata* differed in their proline response to elevation. The increased proline in *A. marina* relatively to the unchanged level in *K. obovata* reflected the stronger resistance of *A. marina* to waterlogging. The proline response of *A. marina* was in favor of osmotic regulation under relatively increased salt stress and boosted the capacity for free radical scavenging. Additionally, with more energy stored in proline, *A. marina* can repair stress-induced damage more efficiently than *K. obovata* when free from waterlogging.

CONCLUSIONS

According to the experimental results and field observations, it can be concluded that *Avicennia marina* saplings are highly tolerant to prolonged waterlogging and the optimal elevation for *A. marina* restoration is the middle elevation with an inundation time of 8 h d^{-1}. In addition, growth of *A. marina* saplings at the lower elevation is not significantly restrained, which means that the lowest elevation set in our study did not reach the critical level of *A. marina* saplings. Therefore, no action on elevation is needed for successful *A. marina* restoration in this region. *Kandelia obovata*, on the other hand, is more

sensitive to waterlogging. The best performance of *K. obovata* occurred at the highest elevation, and thus the optimal elevation for *K. obovata* restoration should be where the inundation time is <6 h d^{-1}. Increased inundation time beyond this level compromises the growth of *K. obovata* saplings. Hence, for successful *K. obovata* restoration, these abandoned crab-culture ponds need to be reconstructed to lift the intertidal elevation up to at least 375 cm above the lowest sea level of Xiamen (the middle elevation in this study).

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