

Distinct responses of growth and respiration to growth temperatures in two mangrove species

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- **Background and Aims** Mangrove plants are mostly found in tropical and sub-tropical tidal flats, and their limited distribution may be related to their responses to growth temperatures. However, the mechanisms underlying these responses have not been clarified. Here, we measured the dependencies of the growth parameters and respiration rates of leaves and roots on growth temperatures in typical mangrove species.
- **Methods** We grew two typical species of Indo-Pacific mangroves, *Bruguiera gymnorhiza* and *Rhizophora stylosa*, at four different temperatures (15, 20, 25 and 30 °C) by irrigating with fresh water containing nutrients, and we measured growth parameters, chemical composition, and leaf and root O₂ respiration rates. We then estimated the construction costs of leaves and roots and the respiration rates required for maintenance and growth.
- **Key Results** The relative growth rates of both species increased with growth temperature due to changes in physiological parameters such as net assimilation rate and respiration rate rather than to changes in structural parameters such as leaf area ratio. Both species required a threshold temperature for growth (12.2 °C in *B. gymnorhiza* and 18.1 °C in *R. stylosa*). At the low growth temperature, root nitrogen uptake rate was lower in *R. stylosa* than in *B. gymnorhiza*, leading to a slower growth rate in *R. stylosa*. This indicates that *R. stylosa* is more sensitive than *B. gymnorhiza* to low temperature.
- **Conclusions** Our results suggest that the mangrove species require a certain warm temperature to ensure respiration rates sufficient for maintenance and growth, particularly in roots. The underground temperature probably limits their growth under the low-temperature condition. The lower sensitivity of *B. gymnorhiza* to low temperature shows its potential to adapt to a wider habitat temperature range than *R. stylosa*. These growth and respiratory features may explain the distribution patterns of the two mangrove species.

Key words: Acclimation, growth, mangroves, O₂ respiration, respiration cost, temperature.

INTRODUCTION

Mangrove plants are mostly found in tropical and sub-tropical tidal flats (Tomlinson, 1986; Spalding *et al.*, 2010). The particularity of their habitats has drawn much interest from plant biologists. On the basis of their global distribution pattern, Duke *et al.* (1998) suggested that mangroves occur in habitats where the mean winter temperature of the water surface is >20 °C. Instantaneous extreme freezing events may determine the latitudinal range limit of the mangrove *Avicennia germinans* (L.) L. in coastal Louisiana, USA (Pickens and Hester, 2011; Osland *et al.*, 2014, 2015, 2017). The speed at which cultivated seedlings of *Rhizophora stylosa* Griff. grow increases as the temperature rises from 15 to 30 °C, and their photosystem II is photoinhibited at temperatures <20 °C (Akaji *et al.*, 2019). Although a warm temperature (almost above 20 °C) is clearly needed for the maintenance and growth of mangrove plants, the mechanisms underlying these phenomena have not been clarified.

Respiratory ATP is used for maintenance and growth requirements such as protein turnover, maintenance of solute concentration gradients across membranes, synthesis of new structures, nutrient uptake, phloem loading and nitrogen (N) assimilation in

plants (de Wit *et al.*, 1970; Amthor, 1989; Thornley and Johnson, 1990; O'Leary *et al.*, 2019). Growth temperature affects the maintenance and growth processes and respiratory O₂ consumption. Therefore, growth and respiration responses to temperature are closely related to each other. For example, changes in root temperature affect the rates of respiration and nutrient uptake in the roots, and whole-plant growth and nutrient demand change to adjust to the nutrient uptake capacity (Chapin, 1974; Chapin and Bloom, 1976; Clarkson *et al.*, 1992). Therefore, clarification of the temperature dependencies of respiration rates in mangroves and their link to growth properties would improve our understanding of the temperature limitation of growth of mangrove plants and how they acclimate to various growth temperatures.

Responses of respiration rates to changing temperatures vary among plant functional types and among their biomes. Meta-analyses of leaf CO₂ respiration rates (i.e. CO₂ emission rates) suggest that these rates under growing temperature are higher in plants from warm climates than in those from cold climates (Wright *et al.*, 2005; Atkin *et al.*, 2015). The warm habitats of mangroves raise the question of whether these plants require a comparatively high respiratory ATP (i.e. high O₂ respiration rate) for their maintenance and growth. Previous studies reported that

leaf and root respiration rates of mangrove species in the genera *Avicennia*, *Bruguiera* and *Rhizophora* lie within the range of 1–6 nmol O₂ or CO₂ g⁻¹ s⁻¹ (Burchett *et al.*, 1984; Hovenden *et al.*, 1995; Lovelock *et al.*, 2006; Atreya and Bhargava, 2008). This range is lower than that of terrestrial plants, i.e. 2–16 nmol O₂ g⁻¹ s⁻¹ (Zha *et al.*, 2001; van Iersel, 2006; Laureano *et al.*, 2008, 2013; Hernández-Montes *et al.*, 2019). Relative growth rates (RGRs) of mangrove plants range from 2 to 6 mg g⁻¹ d⁻¹ under fresh or saline water conditions (Lin and Sternberg, 1993; Farnsworth *et al.*, 1996; Ball *et al.*, 1997; Ellison and Farnsworth, 1997; López-Hoffman *et al.*, 2006). Because RGRs are 100–400 mg g⁻¹ d⁻¹ in herbaceous species and 10–150 mg g⁻¹ d⁻¹ in terrestrial tree species (Poorter and Garnier, 2007), the RGRs of mangrove plants are lower than those of other plants even under non-saline conditions. Even though low rates of growth and respiration appear to be characteristics of mangrove plants, the relationship between growth and respiration rates and their dependencies on growth temperatures are still unknown. Furthermore, most of the above studies on mangroves examined either leaf or root respiration rates, whereas both organs should be analysed to relate growth and maintenance responses to changing temperature. Previously, we found that temperature dependencies of CO₂ respiration rates of stems and roots differed from those of leaves in *R. stylosa*, suggesting that responses of the CO₂ respiration rate to long-term changes in growth temperature may differ between non-assimilatory and assimilatory organs in this species (Akaji *et al.*, 2019).

Here, we focused on two typical Indo-Pacific mangrove species, *Bruguiera gymnorhiza* (L.) Lam. and *R. stylosa*. Both belong to the family Rhizophoraceae, but the former grows in a wide range of western Indo-Pacific areas, whereas the latter does not grow in eastern and southern Africa or in the Middle East (Spalding *et al.*, 2010). The temperature range of the habitats is wider in *B. gymnorhiza* than in *R. stylosa* (Supplementary data Fig. S1), suggesting differences in the temperature dependencies of their growth and respiration rates.

We aimed to compare the dependencies of the growth parameters and O₂ respiration rates of leaves and roots on growth temperatures in these two species. We hypothesized that: (1) the two mangrove species have adapted to warm conditions to ensure an O₂ respiration rate sufficient for their maintenance and growth; (2) temperature dependencies of growth parameters and O₂ respiration rate are linked between leaves and roots; (3) temperature dependencies of growth parameters and O₂ respiration rate differ between the two species; and (4) *B. gymnorhiza* has a wider potential range of growth temperatures than *R. stylosa*.

We grew seedlings at four temperatures, measured growth parameters, chemical compositions, and leaf and root O₂ respiration rates, and estimated the construction costs and respiration rates for maintenance and growth. We examined the relationships between growth parameters and O₂ respiration rates, and discuss the implications in terms of warm habitats of the mangrove species.

MATERIALS AND METHODS

Plant materials and growth conditions

For several months each year, *B. gymnorhiza* and *R. stylosa* produce viviparous seedlings (diaspores) with extended

hypocotyls; these diaspores drop from the mother trees when the diaspore length becomes 20–25 cm. In this study, diaspores of *B. gymnorhiza* and *R. stylosa* (200 of each) were collected from at least 30 mature trees on Iriomote Island (24°17'33"N, 123°51'43"E), one of the southernmost islands of Japan, during the fruiting season (18 January 2018 for *B. gymnorhiza* and 12 July 2017 for *R. stylosa*). According to the Japan Meteorological Agency, the mean monthly precipitation and temperature was 220 mm and 28.0 °C, respectively, in summer (from July to August), and 159 mm and 19.2 °C, respectively, in winter (from November to February) (<https://www.data.jma.go.jp/obd/stats/data/mdrr/index.html>). The collected diaspores were planted in a tray filled with sand (Imai Co. Ltd, Tsukuba, Japan) in a glasshouse (air temperature, 25 °C; relative humidity, 70 %) and watered twice a day. After about 6 months, 100 seedlings were randomly selected and transplanted individually into polycarbonate pots (159 mm diameter; 246 mm depth) with sand. From that time point, we started fertilization with 1:2000-diluted nutrient solution (NO₃⁻, 2.34 mM; NH₄⁺, 0.62 mM; PO₄⁻, 0.50 mM; K, 2.86 mM; Ca, 1.04 mM; Na, 0.13 mM; Mg, 0.42 mM; Fe, 0.01 mM) (Hyponex Japan, Osaka, Japan) twice a day until the end of the cultivation period. There was a drain hole at the bottom of the pots, and 1 L of nutrient solution was showered on each seedling per day. A week after transplantation, 48 seedlings were randomly selected, and 12 seedlings of each species were transferred into four sunlit growth chambers (Koito Electric Industries, Shizuoka, Japan). Each growth chamber was a cube with 2 m sides. The top, west, south and east sides of each chamber were made of glass, and the four chambers were lined up facing south in a sunlit glasshouse in which air temperature was controlled at 27 °C. The double glasshouse structure alleviated the variation of air temperature within each chamber (Supplementary data Fig. S2). The air temperature of the chambers was set at 15, 20, 25 and 30 °C, and humidity was set at 70 %. During the dark period, the air temperature was controlled at the pre-set temperature, whereas it was raised by up to about 5 °C during the light period depending on the solar radiation (Supplementary data Figs S3 and S4). We calculated the regression equations of the air temperatures in the growth chambers against the outside solar radiation, and used them to estimate the mean air temperatures in the growth chambers during the cultivation period. The estimated mean air temperatures in the growth chambers during the cultivation were 15.4, 21.8, 26.6 and 31.7 °C for the four chambers containing *B. gymnorhiza*, and 15.2, 21.5, 26.3 and 31.4 °C for those containing *R. stylosa* (Supplementary data Fig. S5). We used the estimated mean air temperature in each growth chamber during the cultivation rather than the pre-set temperature for all data analyses on growth parameters. Standard deviations of air temperature, light intensity and humidity measurements within the chambers were 0.4 °C, 12.8 µmol m⁻² s⁻¹ and 1.6 %, respectively, and standard deviations of light intensity and humidity among the chambers were 13.2 µmol m⁻² s⁻¹ and 1.8 %, respectively (Supplementary data Fig. S3). The light intensity was affected by weather conditions, but neither the light intensity nor the humidity differed significantly among chambers (Supplementary data Table S1). Air temperature, light intensity and humidity did not differ significantly within a chamber (Supplementary data Table S2). The temperatures at leaves and roots were not significantly different (Supplementary data Fig. S6). Plant heights on the initial

day of the cultivation were 27.8 ± 2.53 cm (mean $\pm$ s.d.) for *B. gymnorhiza*, and 29.4 ± 2.45 cm for *R. stylosa*. The positions of the pots were rotated within each growth chamber twice a week. The plants grown in the chambers that were set at 15, 20, 25 and 30 °C are referred to as 15 °C-, 20 °C-, 25 °C- and 30 °C-growth plants, respectively. The set growth temperature is referred to as the growth condition (e.g. 15 °C growth condition).

Measurement of growth rate, biomass allocation and N uptake rate

On the initial day and 56th day of the cultivation, we sampled seedlings of both species to analyse growth. During the 56 d cultivation period, both species were in the juvenile phase, and developed 2–6 leaves; from four to 8–10 leaves in *B. gymnorhiza* and from four to 6–8 leaves in *R. stylosa*. Neither species flowers during the juvenile phase, which lasts for 6–8 years. No seedlings showed dead leaves during the 56 d cultivation period. The RGR ($\text{g g}^{-1} \text{d}^{-1}$), net assimilation rate (NAR; $\text{g m}^{-2} \text{d}^{-1}$) and leaf area ratio (LAR; $\text{m}^2 \text{g}^{-1}$) were determined for individual plants at each growth temperature according to the following equations (Lambers *et al.*, 2008).

$$\text{RGR} = \frac{1}{W} \frac{dW}{dt} = \frac{\ln W_f - \ln W_i}{t_f - t_i} \quad (1)$$

$$\text{NAR} = \frac{1}{A} \frac{dW}{dt} = \frac{W_f - W_i}{A_f - A_i} \frac{\ln A_f - \ln A_i}{t_f - t_i} \quad (2)$$

$$\text{LAR} = \text{RGR}/\text{NAR} \quad (3)$$

where W_i and W_f are total dry mass (g), and A_i and A_f are leaf area (m^2) on the initial day (t_i) and the final day of cultivation (t_f), respectively.

Leaf mass ratio (LMR; g g^{-1}), root mass ratio (RMR; g g^{-1}) and leaf mass per unit leaf area (LMA; g m^{-2}) were determined for individual seedlings from the dry weights of the whole plant, all leaves, all roots and the total area of all leaves on the final day of cultivation (Lambers *et al.*, 2008).

When seedlings were individually transplanted into pots, another eight seedlings per species were randomly selected from the tray, and the fresh weights of the whole seedlings and of their leaves, stems and roots were determined. All leaves from each of the eight seedlings were scanned, and their areas were measured by ImageJ software (Abramoff *et al.*, 2004). The leaves, stems and roots were then dried at 80 °C until their weight became constant; this final weight was classed as the dry weight. The mean ratios of their dry weight to fresh weight, and the dry weight of each of their organs were used to estimate the initial dry weight of each organ of the pot seedlings in the cultivation experiment. At the end of the 56 d cultivation period, five seedlings per species in each chamber were randomly selected and divided into leaves, stems and roots, and used to measure the O_2 respiration rate. The leaf area of each seedling was then measured as above and all organ samples were dried at 80 °C until their weight became constant, and the dry weight was measured.

Subsequently, the samples were ground into fine powder, 10 mg of each sample was wrapped in tin foil, and N and

carbon (C) contents were measured with an elemental analyser (Flash EA 1112, Thermo Electron Corp., Minneapolis, MN, USA). Each sample was measured three times and the mean value was determined. The C contents were used as organic C contents (C_{org}). The net rate of N uptake per unit root dry mass (NNUR; $\text{nmol N g}^{-1} \text{s}^{-1}$) was determined for each seedling as follows (White, 1972):

$$\text{NNUR} = \frac{\ln W_{\text{rootf}} - \ln W_{\text{rooti}}}{t_f - t_i} \times \frac{N_f - N_i}{W_{\text{rootf}} - W_{\text{rooti}}} \quad (4)$$

where W_{rooti} and W_{rootf} are the root dry mass (g) at the initial day (t_i) and final day (t_f), respectively; and N_i and N_f are the total N amounts in the plant (nmol) at t_i and t_f , respectively.

Measurement of oxygen respiration rates

At the end of the 56 d cultivation period, O_2 respiration rates of leaves and roots (R ; $\text{nmol O}_2 \text{g d. wt}^{-1} \text{s}^{-1}$) were measured individually in five randomly selected seedlings per species as follows. Leaves and roots were detached into separate 50 mL glass vials, and O_2 consumption rates were measured with a fluorescence O_2 sensor (FDO 925, Xylem Analytics, Weilheim, Bavaria, Germany). During measurement, the samples were kept at 15, 20, 25 or 30 °C to match their growth temperature. Measurements were conducted in a gaseous phase with all leaves of one seedling, and in a liquid phase with about half of the root system of one seedling. For root measurements, each vial was filled with air-saturated nutrient solution (1:2000-diluted Hyponex) containing 50 mM MES buffer (pH 6.5). A constant rate of O_2 uptake was recorded at the corresponding temperature for 15 min. Each sample was retrieved from its vial, dried at 80 °C until the weight became constant and then weighed to give the dry weight.

The O_2 respiration rates for the growth of leaves and roots (R_g ; $\text{nmol O}_2 \text{g d. wt}^{-1} \text{s}^{-1}$) were obtained by multiplying RGR by the regression coefficient for growth (g ; $\text{mol C g d. wt}^{-1}$) of each organ, assuming that the respiratory quotient $\text{RQ} = 1$ (Hachiya and Noguchi, 2008). The RQ has been reported to be around 1 in *Bruguiera* and *Rhizophora* species (Chapman, 1962; Brown *et al.*, 1969), and in many other plants (Lambers and Oliveira, 2019). RGR and g at 15, 20, 25 and 30 °C were calculated by using fitted curves for measured RGR or g vs. the estimated mean growth temperature. O_2 respiration rate for leaf maintenance (R_m ; $\text{nmol O}_2 \text{g d. wt}^{-1} \text{s}^{-1}$) and O_2 respiration rate for root maintenance and N uptake ($R_m + R_u$; $\text{nmol O}_2 \text{g d. wt}^{-1} \text{s}^{-1}$, where R_u is the root O_2 respiration rate for N uptake) were obtained by subtracting R_g from the O_2 respiration rate for each organ. R_u ($\text{nmol O}_2 \text{g d. wt}^{-1} \text{s}^{-1}$) was obtained by multiplying the specific cost of nitrate uptake (c_u ; $\text{mol O}_2 \text{mol}^{-1} \text{NO}_3^{-1}$) by NNUR and the ratio of nitrate N to total N in the nutrient solution (mol mol^{-1}). The ratio was set to 0.75 because the estimated concentration of nitrate N was 2.34 mmol L^{-1} and that of total N was 3.13 mmol L^{-1} in 1:2000-diluted Hyponex solution. NNUR values at 15, 20, 25 and 30 °C were calculated by using fitted curves of the measured NNUR vs. the estimated mean growth temperature. Theoretical R_u can be obtained under several assumptions from the following equations (Bouma *et al.*, 1996; Scheurwater *et al.*, 1999; Kurimoto *et al.*, 2004a).

$$R_u = \frac{I}{I-E} \times c_u \times \text{NNUR} \times a \quad (5)$$

$$c_u = \frac{\frac{H^+}{I_j} \times M_j}{\frac{H^+}{P} \times \frac{P}{O_2}} = \frac{2}{\frac{P}{O_2}} \quad (6)$$

where I is the influx of nitrate into the cytoplasm, E is the efflux (or leak) of nitrate out of the cytoplasm (Scheurwater *et al.*, 1999), a is the proportion of nitrate N in the total N in the nutrient solution (mol mol^{-1}), H^+/I_j is the number of protons required for a membrane symport of nitrate ($2 \text{ mol H}^+ \text{ mol}^{-1} \text{ NO}_3^-$; Crawford and Glass, 1998), M_j is the number of membranes to be crossed actively ($= 1$; the plasma membrane), H^+/P is the amount of protons pumped across the plasma membrane by the H^+ -ATPase per hydrolysis of one ATP to ADP ($= 1 \text{ mol H}^+ \text{ mol}^{-1} \text{ ATP}$; Crawford and Glass, 1998), and P/O_2 is the efficiency of oxidative phosphorylation [29/6 when only the cytochrome pathway (CP) is engaged, and 11/6 when only the alternative pathway (AP) is engaged; Amthor, 1994]. Therefore, (1) efflux of nitrate and (2) electron partitioning to AP (τ_a) have positive effects on R_u . For example, when no efflux of nitrate occurs ($E = 0$) and all electrons flow to the CP ($P/O_2 = 29/6$), $\frac{I}{I-E} \times c_u$ will be 0.41, and when nitrate efflux is 60 % ($E = 0.6$) and electrons flow equally to the CP and AP ($P/O_2 = 20/6$), $\frac{I}{I-E} \times c_u$ will be 1.50. We estimated the R_u in the theoretical range of $\frac{I}{I-E} \times c_u$ values as being 0.41–1.50 $\text{mol O}_2 \text{ mol}^{-1} \text{ NO}_3^-$.

Determination of protein contents of leaves and roots

At the end of the cultivation experiment, the leaves and roots of each seedling were collected and approx. 200 mg per seedling was stored at -80°C until protein extraction. Each sample was ground with a mortar and pestle in buffer containing 2 % (w/v) SDS 62.5 mM tris(hydroxymethyl)aminomethane-HCl (pH 6.8), 50 mM dithiothreitol, 7.5 % (v/v) glycerol, 0.01 % (w/v) bromophenol blue and a protease inhibitor tablet (Roche Diagnostics, Mannheim, Germany). The homogenate was heated at 100°C for 5 min and centrifuged at $15\,000\text{ g}$ for 10 min. Protein content of the supernatant was measured according to the method of Peterson (1977) with bovine serum albumin as the standard.

Estimation of construction costs and regression coefficients for growth of leaves and roots

The construction cost (CC; the amount of glucose required to produce 1 g of biomass, g glucose g^{-1}) of leaves and roots was determined as follows (Poorter, 1994; Hachiya and Noguchi, 2008):

$$\text{CC} = (-1.041 + 5.077 \times C_{\text{org}}) \times (1 - m) + 5.325 \times N_{\text{org}} \quad (7)$$

where m is mineral content, N_{org} is organic N content (g g d. wt^{-1}), and C_{org} is organic C content (g g d. wt^{-1}).

Mineral content was obtained by multiplying ash content ($\text{g ash weight g d. wt}^{-1}$) by 0.67. This coefficient has been used for

many plant species (e.g. Vertregt and Penning de Vries, 1987; Hachiya and Noguchi, 2008). Dried samples were combusted at 550°C for 5 h and the ash was weighed. Organic N content was obtained by subtracting nitrate content from total N content; nitrate content of dried samples was obtained by the colorimetric method (Cataldo *et al.*, 1975) as follows. Each dried sample (10 mg) was extracted with 1 mL of distilled water at 100°C for 30 min; 0.2 mL of the extract was pipetted into a 50 mL flask and mixed with 0.8 mL of 5 % (w/v) salicylic acid that was dissolved in concentrated H_2SO_4 . After 20 min at room temperature, 19 mL of 2 N NaOH was added, and the mixture was cooled to room temperature for 10 min. Absorbance at 410 nm was measured with a spectrophotometer (U-1100, Hitachi High-Tech Corporation, Tokyo, Japan).

The regression coefficient for growth (g ; $\text{mol C g d. wt}^{-1}$) of leaves and roots was determined as follows (Hachiya and Noguchi, 2008):

$$g = \left(\text{CC} \times \frac{6}{180.15} \right) - \left(\frac{C_{\text{org}}}{12.01} \right) \quad (8)$$

where 180.15 is the molecular weight of glucose (g mol^{-1}), 12.01 is the atomic weight of C (g mol^{-1}) and 6 is the number of C atoms in one glucose.

Statistical analysis

Growth temperature dependencies of the parameters, and LMA dependencies of leaf CC were fitted by second-order polynomial regression with a 95 % confidence interval. Linear regression was used to examine relationships between RGR and NAR and between RGR and LAR. For comparison of growth, biomass allocation and morphology among species and set growth conditions, two-way analysis of variance (ANOVA) was conducted after the normality of the raw data was confirmed by a normality test. For comparison of O_2 respiration rates, construction costs and regression coefficients for growth and protein contents among species, organs and assigned growth temperatures, a multifactor ANOVA was used after normality of the raw data was confirmed as above. Treatments were compared by conducting a Tukey's multiple-comparison test using the 'glht' function in the 'multcomp' package (Hothorn *et al.*, 2008) of R v.3.6.2 software (R Core Team, 2017). A type I error rate ($P < 0.05$) was considered significant.

RESULTS

Dependencies of growth parameters on growth temperatures in two mangrove species

We examined plant growth at four different temperatures (15, 20, 25 and 30°C) for five plants of each species. On the initial day of the cultivation, the dry weight of the whole plant (mean $\pm$ SD) was $9.02 \pm 1.42\text{ g}$ for *B. gymnorhiza* and $13.69 \pm 1.39\text{ g}$ for *R. stylosa*. On the final day of the 56 d cultivation period, the mean whole dry weight was 9.61 g (15°C), 10.53 g (20°C), 12.13 g (25°C) and 12.88 g (30°C) for *B. gymnorhiza* and 12.60 g (15°C), 14.87 g (20°C), 15.29 g (25°C) and 15.08 g (30°C) for *R. stylosa*. The RGR of the whole plant (total RGR) differed

significantly between the species and among growth temperatures (Fig. 1A; both $P < 0.001$, ANOVA in Supplementary data Table S3). Total RGR was significantly higher in *B. gymnorhiza* than in *R. stylosa* at 20–30 °C ($P < 0.05$, Tukey's multiple comparison test in Supplementary data Table S4), and it increased with growth temperature in both species (Supplementary data Table S4, S5). In the ANOVA, the interaction term between species and growth temperature was significant, indicating that the temperature dependencies of total RGR differed between the two species ($P < 0.05$, Supplementary data Table S3); the increment of total RGR against growth temperature was higher in *B. gymnorhiza* than in *R. stylosa*. Total RGR is determined by NAR and LAR ($\text{RGR} = \text{NAR} \times \text{LAR}$). NAR differed significantly between the species (Fig. 1D; $P < 0.01$, Supplementary data Table S3) and among growth temperatures ($P < 0.001$, Supplementary data Table S3). There was no significant interaction between the effects of species and growth temperature on NAR (Supplementary data Table S3). Despite the significant result by ANOVA, the interspecific differences were not significant at any growth temperature in the Tukey's multiple comparison test (Supplementary data Table S4). Values of total RGR and NAR were negative for four out of five *R. stylosa* plants under the 15 °C growth condition. Total RGR and NAR were strongly correlated with each other in both species (both $R^2 = 0.98$), but the regression coefficient in *B. gymnorhiza* was 2.19 times that in *R. stylosa* (Fig. 2A, B). The LAR differed significantly between the species and among growth temperatures (Fig. 1E; $P < 0.001$, Supplementary data Table S3). There was no significant interaction between the effects of species and growth temperature on LAR (Supplementary data Table S3). Although LAR significantly increased with growth temperature, the temperature dependencies of LAR were small in both species (Fig. 1E; Supplementary data Table S5).

The above results suggest that the differences in total RGR among growth temperatures can be explained by those in NAR in both species. In *R. stylosa*, the negative values of total RGR of 15 °C-growth plants may be due to the negative values of NAR, which suggests that photosynthesis and/or respiration are not well regulated in *R. stylosa* grown under this low temperature. The fitted curves for measured total RGR vs. estimated mean growth temperature indicated that total RGR became zero at 12.2 °C in *B. gymnorhiza* and at 18.1 °C in *R. stylosa* (Table 1; Supplementary data Table S5).

The interspecific difference in total RGR could be explained mainly by LAR being significantly higher in *B. gymnorhiza* than in *R. stylosa* (Fig. 1E; $P < 0.001$, Supplementary data Tables S3 and S4). LAR is determined by specific leaf area, SLA, and LMR ($\text{LAR} = \text{SLA} \times \text{LMR}$). We compared LMR and LMA, the reciprocal of SLA. LMR differed significantly between the species and among growth temperatures (Fig. 1G; $P < 0.001$, Supplementary data Table S3). There was no significant interaction between the effects of species and growth temperature on LMR (Supplementary data Table S3). LMR was higher in *B. gymnorhiza* than in *R. stylosa* under 15 and 20 °C growth conditions (Supplementary data Table S4). In both species, LMR increased significantly as growth temperature increased from 15 to 25 °C, and was saturated under the 25 and 30 °C growth conditions (Fig. 1G; Supplementary data Tables S4 and S5).

The LMA differed significantly between the species (Fig. 1I; $P < 0.001$, Supplementary data Table S3) and among growth temperatures ($P < 0.05$, Supplementary data Table S3). There was no significant interaction between the effects of species and growth temperature on LMA. Although LMA slightly decreased with growth temperature in both species, the decrease was small (Fig. 1I; Supplementary data Table S5) and was not significant by Tukey's multiple comparison test (Supplementary data Table S4). LMA was significantly higher in *R. stylosa* than in *B. gymnorhiza* under all growth conditions, probably because *R. stylosa* has thicker leaves than *B. gymnorhiza* (Fig. 1I; Supplementary data Table S4); the extent of interspecific difference in LMA was higher than that in LMR.

Regarding N acquisition, RMR differed significantly between the species and among the growth temperatures (Fig. 1H; $P < 0.001$, Supplementary data Table S3). The interaction between species and growth temperature was significant, indicating that the temperature dependencies of RMR differed between the two species ($P < 0.01$, Supplementary data Table S3). In the 15–25 °C growth conditions, RMR was significantly larger in *B. gymnorhiza* than in *R. stylosa*; RMR increased with growth temperature in *B. gymnorhiza*, but was almost constant in *R. stylosa* (Supplementary data Tables S4 and S5). The NNUR and N content of the plant (Nc) differed significantly between the species (Fig. 1F, J; $P < 0.01$ for NNUR and $P < 0.001$ for Nc, Supplementary data Table S3) and among growth temperatures ($P < 0.001$ for both NNUR and Nc, Supplementary data Table S3). The NNUR and Nc increased with growth temperature in both species; there was no significant interaction between the effects of species and growth temperature on either parameter (Supplementary data Tables S3 and S5). The interspecies difference was only significant for Nc under the 20 and 30 °C growth conditions in the Tukey's multiple comparison test (Supplementary data Table S4). *Rhizophora stylosa* had fewer roots and thus slower rates of N uptake than *B. gymnorhiza*, especially at the low temperature.

Leaf RGR differed significantly among growth temperatures (Fig. 1B; $P < 0.001$, Supplementary data Table S3), but not between species. There was no significant interaction between the effects of species and growth temperature on leaf RGR (Supplementary data Table S3). The fitted curves for leaf RGR vs. estimated mean growth temperature peaked at 34.5 °C in *B. gymnorhiza* and at 29.3 °C in *R. stylosa*; the peak was estimated to be where the primary differential coefficient (slope) of the fitted curve became zero (Supplementary data Table S5). In these fitted curves, RGR became zero at 11.8 °C in *B. gymnorhiza* and at 14.9 °C in *R. stylosa* (Table 1). Unlike leaf RGR, root RGR differed significantly between the species as well as among growth temperatures (Fig. 1C; both $P < 0.001$, Supplementary data Table S3). Furthermore, for root RGR, the interaction between species and growth temperature was significant, indicating that the temperature dependencies of root RGR differed between the two species ($P < 0.01$, Supplementary data Table S3). Root RGR increased with growth temperatures across the entire range in *B. gymnorhiza*, but was saturated when the plants were cultured under 25 or 30 °C growth conditions in *R. stylosa*. Root RGR was significantly higher in *B. gymnorhiza* than in *R. stylosa* when the plants were cultured under ≥ 20 °C growth conditions (Supplementary data

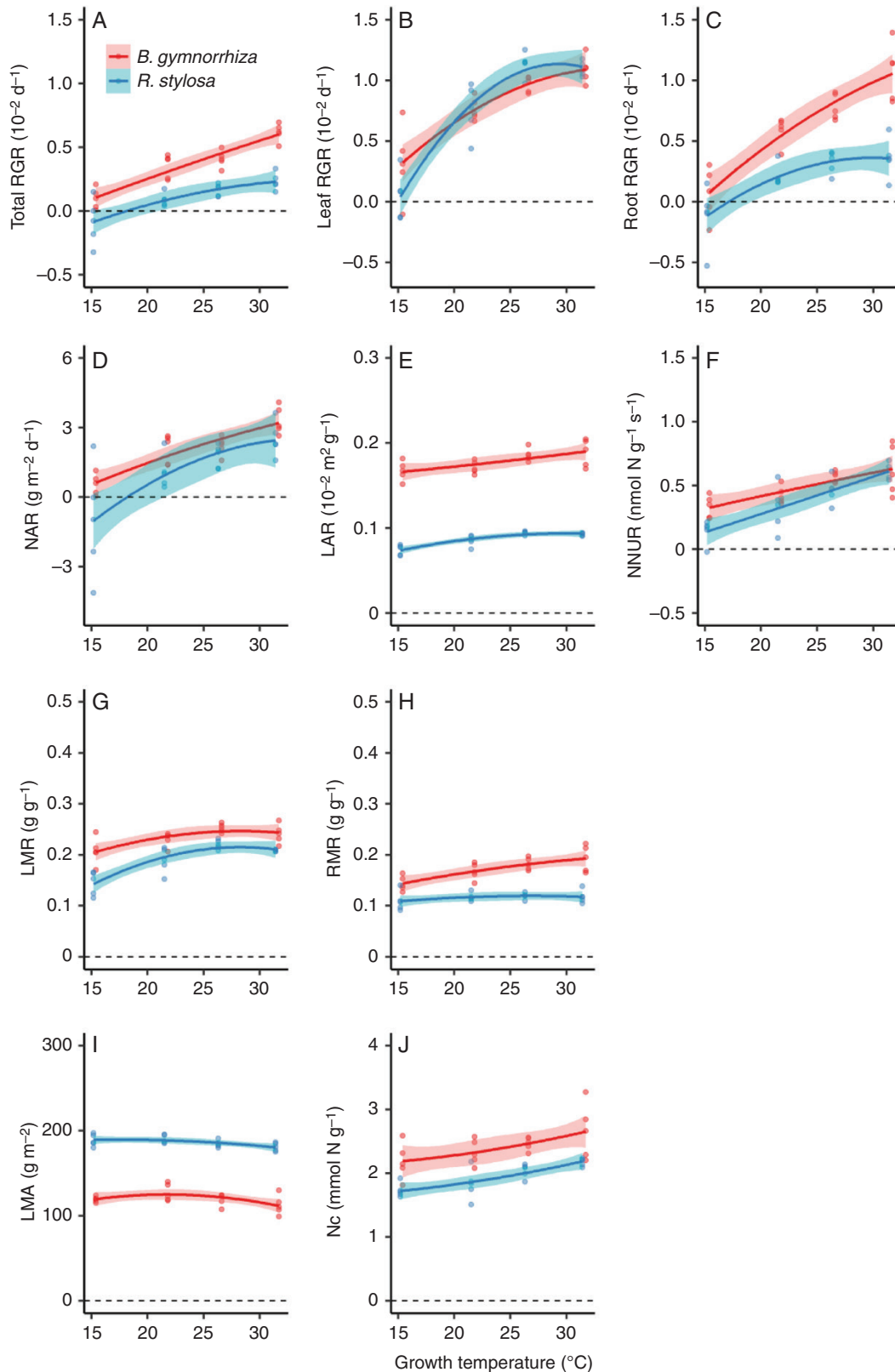


FIG. 1. Growth parameters of *Bruguiera gymnorrhiza* and *Rhizophora stylosa* at different growth temperatures. (A) Total relative growth rate (RGR), (B) leaf RGR, (C) root RGR, (D) net assimilation rate, (E) leaf area ratio, (F) net rate of nitrogen uptake per unit root dry mass, (G) leaf mass ratio, (H) root mass ratio, (I) leaf mass per unit leaf area and (J) plant nitrogen content at different growth temperatures. The growth temperatures on the x-axis are the estimated mean air temperatures in the growth chambers during the cultivation experiment. Solid lines are fitted curves of the second-order polynomial regression model, and the red and blue shading indicates 95 % confidence intervals. Dashed lines indicate zero on the y-axis. Statistical results and the coefficients and intercepts of the regression model are summarized in [Supplementary data Tables S3–S5](#).

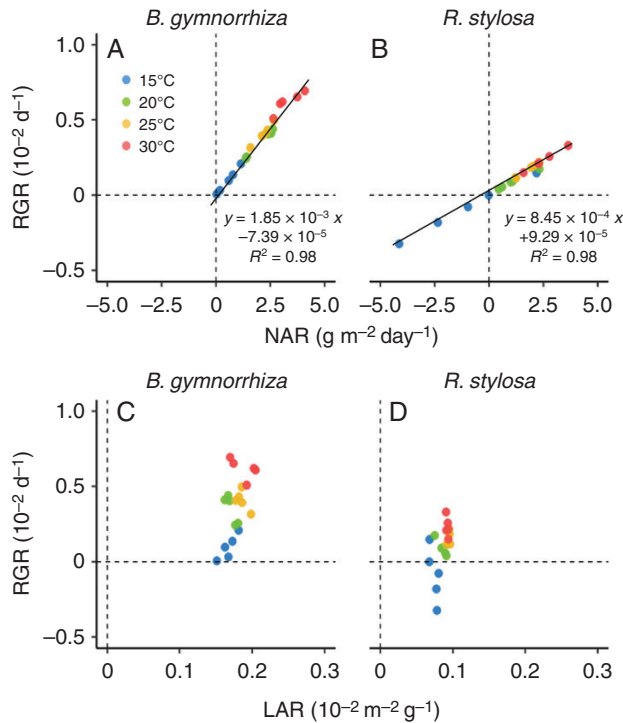


FIG. 2. Relationships between (A, B) total RGR and NAR and (C, D) total RGR and LAR in (A, C) *B. gymnorhiza* and (B, D) *R. stylosa*. Solid lines indicate the fitted linear regression model.

TABLE 1. Growth temperatures at which fitted curves of RGR become zero in whole plants, leaves and roots of *B. gymnorhiza* and *R. stylosa*

	<i>B. gymnorhiza</i>	<i>R. stylosa</i>
Temperature (°C)		
Whole plant	12.2	18.1
Leaf	11.8	14.9
Root	14.5	17.1

Table S4). In the fitted curves for root RGR vs. estimated mean growth temperature (Supplementary data Table S5), root RGR became zero at 14.5 °C in *B. gymnorhiza* and at 17.1 °C in *R. stylosa* (Table 1).

Construction cost and protein content of leaves and roots

The above results indicate that biomass allocation to leaves and roots (i.e. LMR and RMR, respectively) was larger in *B. gymnorhiza* than in *R. stylosa*. We therefore determined the chemical compositions and estimated the construction costs of leaves and roots in both species.

The C content differed significantly between the species (Fig. 3A, B; $P < 0.001$, ANOVA), between organs ($P < 0.001$, ANOVA) and among growth temperatures ($P < 0.01$, ANOVA) (Supplementary data Table S3). The ANOVA results showed a significant three-way interaction among species, organs

and growth temperature, indicating that the temperature dependencies of C content differed among the combinations of species and organs ($P < 0.01$, Supplementary data Table S3). Leaf C content was significantly lower in *B. gymnorhiza* than in *R. stylosa* at 15 and 20 °C growth temperatures (Fig. 3A; Supplementary data Table S4), but root C content did not differ significantly between the species (Fig. 3B; Supplementary data Table S4). The fitted curves for C content vs. estimated mean growth temperature showed slightly different trends between the leaves of the two species: C content increased with growth temperature at ≥ 20 °C in *B. gymnorhiza* leaves, but slightly decreased with growth temperature in *R. stylosa* leaves. However, C content decreased with growth temperature across the whole temperature range tested in the roots of both species (Fig. 3A, B; Supplementary data Table S5).

The N content differed significantly between the species, between organs and among growth temperatures (Fig. 3C, D; $P < 0.001$, Supplementary data Table S3). Leaf N content was higher in *B. gymnorhiza* than in *R. stylosa* (Fig. 3C; $P < 0.001$, Supplementary data Tables S3 and S4), and was higher in leaves than in roots in both species (Fig. 3C, D; $P < 0.001$, Supplementary data Tables S3 and S4). The interaction between the effects of species and organs on N content was significant, indicating that the difference in N content between leaves and roots differed between the two species ($P < 0.001$, Supplementary data Table S3); this difference was larger in *B. gymnorhiza* than in *R. stylosa*. There was also a significant interaction between the effects of organ and growth temperature on N content, indicating that the temperature dependencies of N content differed between organs ($P < 0.001$, Supplementary data Table S3). In both species, the N content increased with growth temperature in the leaves (Fig. 3C; Supplementary data Table S4), but remained constant in the roots (Fig. 3D; Supplementary data Table S4).

Because most of the N taken up by the plant is used for protein synthesis, we examined the protein contents of leaves and roots. Protein content was higher in the leaves than in the roots in both species (Fig. 3E, F; $P < 0.001$, Supplementary data Tables S3 and S4). There was a significant interaction between species and organ ($P < 0.001$, Supplementary data Table S3), indicating that the difference in protein content between leaves and roots differed between the two species; this difference was higher in *B. gymnorhiza* than in *R. stylosa* (Fig. 3E, F). The interaction between species and growth temperature was also significant ($P < 0.05$, Supplementary data Table S3), indicating that the temperature dependencies of protein content differed between the two species. The fitted curves for protein content vs. estimated mean growth temperature showed that protein content tended to increase with growth temperature in the leaves of *B. gymnorhiza* but to decrease with growth temperature in those of *R. stylosa* (Fig. 3E; Supplementary data Table S5).

Nitrate N content differed between the species (Supplementary data Fig. S7A, B; $P < 0.01$, Supplementary data Table S3), between organs ($P < 0.01$, Supplementary data Table S3) and among growth temperatures ($P < 0.001$, Supplementary data Table S3). The interaction between organs and growth temperature was significant ($P < 0.05$, Supplementary data Table S3), indicating that the temperature dependencies of nitrate N content differed between leaves and roots. Nitrate N content

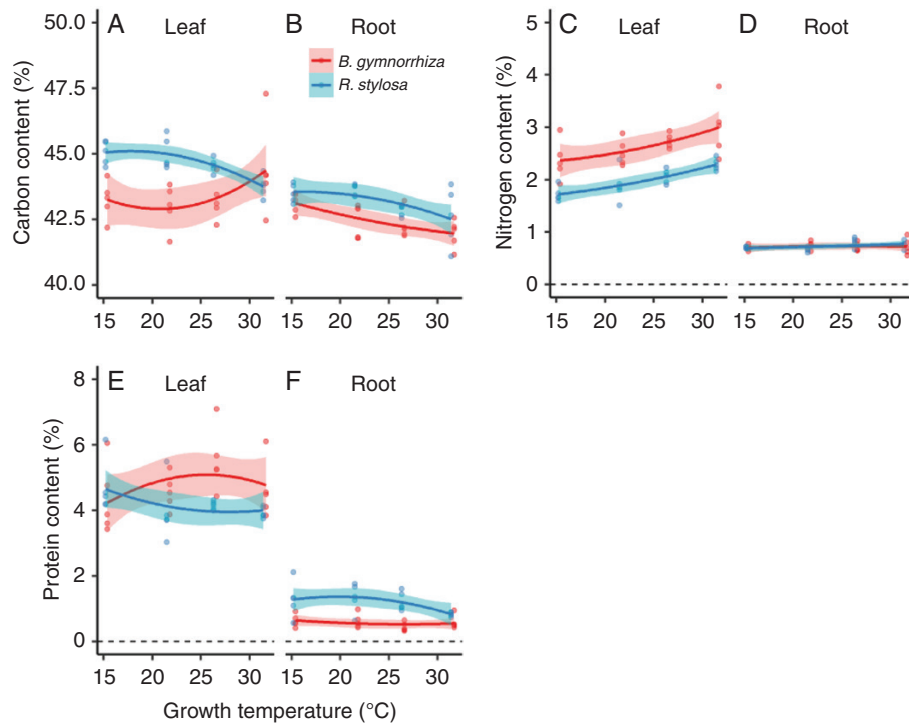


Fig. 3. Contents of (A, B) carbon, (C, D) nitrogen and (E, F) protein in (A, C, E) leaves and (B, D, F) roots of *B. gymnorrhiza* and *R. stylosa*. For other details, see the legend of Fig. 1.

decreased with growth temperature in leaves, but was almost constant in roots (Supplementary data Fig. S7A, B).

Mineral content differed significantly among growth temperatures (Supplementary data Fig. S7C, D; $P < 0.001$, Supplementary data Table S3). The three-way interaction among species, organs and growth temperature was significant ($P < 0.05$, Supplementary data Table S3), indicating that the temperature dependencies of mineral content differed among the combinations of species and organs. Leaf mineral content decreased with growth temperature in *B. gymnorrhiza*, but increased with growth temperature in *R. stylosa* (Supplementary data Fig. S7C, D). Root mineral content was almost constant in the two species.

Using the data on N, C, nitrate and mineral contents and eqn (7), we estimated the CC of leaves and roots. The CC differed significantly between the species (Fig. 4A, B; $P < 0.05$, Supplementary data Table S3) and between organs ($P < 0.001$, Supplementary data Table S3). CC was higher in the leaves than in the roots in both species (Fig. 4A, B; $P < 0.001$, Supplementary data Tables S3 and S4). There was a significant three-way interaction among species, organs and growth temperature ($P < 0.01$, Supplementary data Table S3), indicating that temperature dependencies of CC differed among the combinations of species and organs. The fitted curves of CC vs. estimated mean growth temperature showed that CC increased with growth temperature in the leaves of *B. gymnorrhiza*, but slightly decreased with growth temperature in the leaves of *R. stylosa* and in the roots of both species (Fig. 4A, B; Supplementary data Table S5). In *R. stylosa*, a positive relationship between leaf CC and LMA was observed ($P < 0.001$, Fig. 5).

The regression coefficient for growth, g , calculated using the CC data and eqn (8), differed significantly between organs ($P < 0.001$, Supplementary data Table S3); it was significantly higher in the leaves than in the roots in both species (Fig. 4C, D; Supplementary data Table S4). The three-way interaction among species, organ and growth temperature was significant ($P < 0.001$, Supplementary data Table S3), indicating that the temperature dependencies of g differed among the combinations of species and organs. As observed for CC, the fitted curves of g vs. estimated mean growth temperature showed that g increased with growth temperature in the leaves of *B. gymnorrhiza*, but slightly decreased in the leaves of *R. stylosa* and the roots of both species (Fig. 4C, D; Supplementary data Table S5).

Leaf and root O_2 respiration rates at different growth temperatures

Since we observed differences in growth parameters and their dependencies on growth temperature, we examined leaf and root R, which are related to ATP production for growth, maintenance and N uptake. R significantly differed between species, between organs and among growth temperatures (Fig. 6A, B; $P < 0.001$, Supplementary data Table S3). We observed a significant three-way interaction among species, organs and growth temperature ($P < 0.001$, Supplementary data Table S3), indicating that the temperature dependencies of R differed among the combination of species and organs. Leaf R increased steadily with growth temperature in *B. gymnorrhiza*, but peaked at 25 °C in *R. stylosa* (Fig. 6A; Supplementary

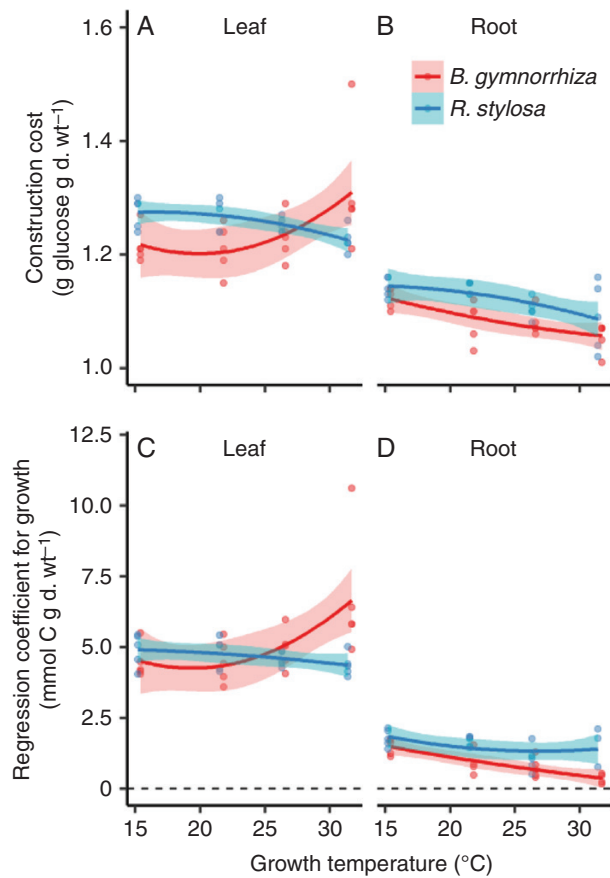


FIG. 4. Construction cost and regression coefficient for growth of (A, C) leaves and (B, D) roots in *B. gymnorrhiza* and *R. stylosa*. For other details, see the legend of Fig. 1.

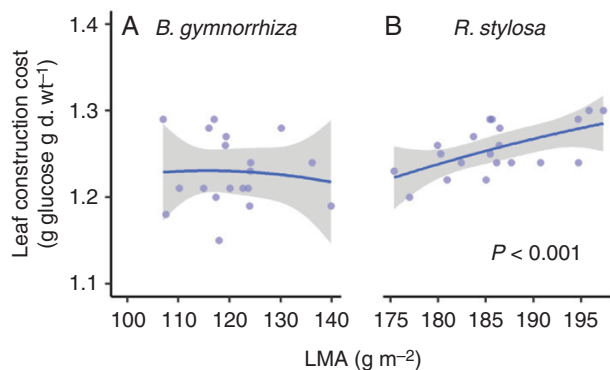


FIG. 5. Relationships between construction cost and leaf mass per unit leaf area of (A) *B. gymnorrhiza* and (B) *R. stylosa*. Solid lines indicate fitted curves of the second-order polynomial regression model, and grey shading indicates the 95% confidence intervals.

data Table S4). The fitted curve of leaf R vs. estimated mean growth temperature (Supplementary data Table S5) peaked at 27.2 °C in *R. stylosa*. The temperature dependencies of leaf R were larger in *B. gymnorrhiza* than in *R. stylosa*, and leaf R in *B. gymnorrhiza* at 30 °C was twice that in *R. stylosa*. In

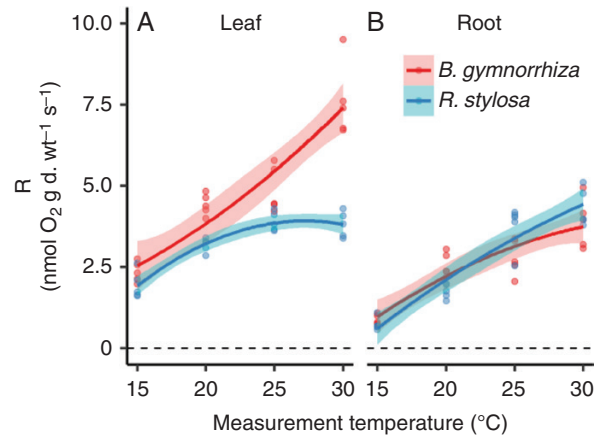


FIG. 6. O_2 respiration rates (R) of (A) leaves and (B) roots of *B. gymnorrhiza* and *R. stylosa*. For other details, see the legend of Fig. 1.

contrast, root R was similar in both species, and increased significantly with growth temperature (Fig. 6B; Supplementary data Table S4). The R values at the temperatures where RGR became zero were 1.89 and 1.91 nmol O_2 g d. wt⁻¹ s⁻¹ in the leaves of *B. gymnorrhiza* and *R. stylosa*, respectively, and 0.84 and 1.28 nmol O_2 g d. wt⁻¹ s⁻¹ in the roots of *B. gymnorrhiza* and *R. stylosa*.

Positive correlations were observed between leaf R and root R in both species (Fig. 7), but the relationship differed between the two species. In *B. gymnorrhiza*, both leaf R and root R increased with growth temperature, but in *R. stylosa* leaf R did not increase from 25 to 30 °C, whereas root R did increase (Fig. 6A, B).

O₂ respiration costs for growth, maintenance and N uptake

Using the data for g, RGR and R, we calculated respiration rates for growth (R_g) and estimated respiration rates for maintenance (R_m) and N uptake (R_u) in roots (Fig. 8). RGR and g at 15, 20, 25 and 30 °C were calculated from the fitted curves of measured RGR or g vs. the estimated mean growth temperature (Figs 1 and 4). In both species, R_g was low, especially in roots (Fig. 8A, B), and thus leaf R_m and root R_m accounted for most of R (Fig. 8C, D). The contribution of R_g to R was <20 % in leaves and <5 % in roots in both species (Fig. 8C, D).

Both leaf R_m and leaf R_g increased steadily with growth temperature in *B. gymnorrhiza*, but were saturated at 25 and 30 °C in *R. stylosa* (Fig. 8A, B). Although both leaf R_m and leaf R_g responded positively to growth temperature, their temperature dependencies differed from each other. Thus, the proportion of R_g in R varied among growth temperatures in both species (Fig. 8C, D). This proportion in leaves increased with growth temperature and was saturated at 25 and 30 °C in *B. gymnorrhiza* and at 20–30 °C in *R. stylosa*, and was higher in *R. stylosa* than in *B. gymnorrhiza* at temperatures of ≥20 °C (Fig. 8C, D). Root R_g was small in both species (Fig. 8).

We estimated R_u using the NNUR data, the assumed c_u values and the ratio of nitrate N to total N in the nutrient solution. R_u increased with growth temperature (Fig. 8A, B) owing to the increase in NNUR with growth temperature. The NNUR data

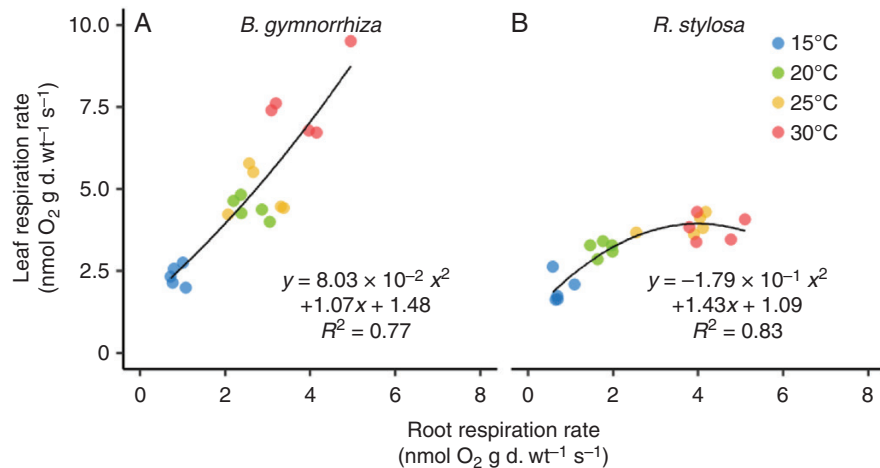


FIG. 7. Relationships between leaf and root O_2 respiration rates of (A) *B. gymnorrhiza* and (B) *R. stylosa*. Solid lines indicate fitted curves of the second-order polynomial regression model.

at 15, 20, 25 and 30 °C were calculated from the fitted curves of measured NNUR vs. the estimated mean growth temperature (Fig. 1F). Irrespective of the assumed values ($\frac{I}{I-E} \times c_u = 0.41$; $E = 0$ and $P/O_2 = 29/6$ in Fig. 8A, or $\frac{I}{I-E} \times c_u = 1.50$; $E = 0.6$ and $P/O_2 = 20/6$ in Fig. 8B), the proportion of R_u in R was 0.1–0.3 and decreased with growth temperature (Fig. 8C, D).

DISCUSSION

Comparison of growth parameters in two mangrove species

In both mangrove species used in this study, growth temperature affected physiological parameters related to growth (NAR and NNUR) rather than structural parameters (LAR and RMR) (Fig. 1). These results are consistent with the reported data on evergreen perennials (Tjoelker *et al.*, 1999; Bruhn *et al.*, 2007). The two species differed in some growth parameters and in their responses to growth temperature. At the same age, *B. gymnorrhiza* had higher leaf and root mass than *R. stylosa* (Fig. 1G, H) and invested more photosynthates into roots at high growth temperature, resulting in higher RGR than in *R. stylosa*.

We also detected a structural difference between the leaves of these species. *Rhizophora stylosa* had higher LMA (i.e. thicker leaves) than *B. gymnorrhiza* (Fig. 1I). This is consistent with the results of Saenger and West (2016), in which SLA of mature trees was higher in *B. gymnorrhiza* than in *R. stylosa* at 13 locations along the Australian coast. A positive relationship between LMA and CC has been reported in deciduous trees due to thick leaves, which have costly cellular components such as lignin (Niinemets, 1999). We found that leaf CC was positively related to LMA in *R. stylosa*, whereas there was no relationship in *B. gymnorrhiza* (Fig. 5). This suggests that a C cost is required to produce the thick leaves of *R. stylosa*. Despite the differences in leaf and root CC between the two mangrove species, the measured leaf and root CCs of both species were within the reported range of temperate terrestrial plants, 1.2–1.9 g glucose g^{-1} (Niinemets, 1999; Martínez *et al.*, 2002a, b; van Iersel, 2006; Laureano *et al.*, 2008, 2013). Therefore, the leaf respiratory features of these two mangrove species are no more costly than those of terrestrial plants.

Plant mass at the end of the 56 d cultivation experiment was higher at higher growth temperatures. Regardless of changes in growth environments such as growth temperature, the RGR is known to decrease as plant mass increases because of ontogenetic drift; the decline in the RGR of tree seedlings with size is concomitant with a decline in the LMR and an increase in the stem mass fraction (Walters *et al.*, 1993; Cornelissen *et al.*, 1996). Although RGR and LMR were rather higher at the higher growth temperatures in which plant mass was higher, we cannot exclude the possibility that RGR at these temperatures was underestimated in our study. If there was ontogenetic drift between the growth temperatures, the difference between low and high growth temperatures may be larger than we observed. In our study, the difference in mean whole dry weight among growth temperatures at the 56th day of cultivation was 3.27 g for *B. gymnorrhiza* and 2.69 g for *R. stylosa*. By using the data for Betulaceae species in Walters *et al.* (1993), we estimate that a 3.5 g plant mass difference may correspond to a difference in RGR of about 0.005 d^{-1} . Betulaceae species with higher RGR tend to have higher ontogenetic drift than those with lower RGR (Walters *et al.*, 1993). The RGRs of Betulaceae species in Walters *et al.* (1993) are about ten times that of the two Rhizophoraceae species in our study, and thus the ontogenetic drift of the Rhizophoraceae species may be smaller than the estimated value.

Different responses of two mangrove species to limiting growth temperatures

Our finding that total, leaf and root RGR reached zero at higher temperatures in *R. stylosa* than in *B. gymnorrhiza* in the fitted curves of RGR vs. estimated mean growth temperature suggests that the seedlings of *R. stylosa* are more sensitive to low temperatures than those of *B. gymnorrhiza*. Our results also suggest that the roots of both species are more sensitive to low temperature than are the leaves, since both species had difficulty in growing roots at below threshold temperatures (i.e. temperature at which RGR reached zero). The O_2 respiration rates of the two mangrove species at the threshold temperatures (1.89 and 1.91 $nmol O_2 g d. wt^{-1} s^{-1}$ in leaves of *B. gymnorrhiza*

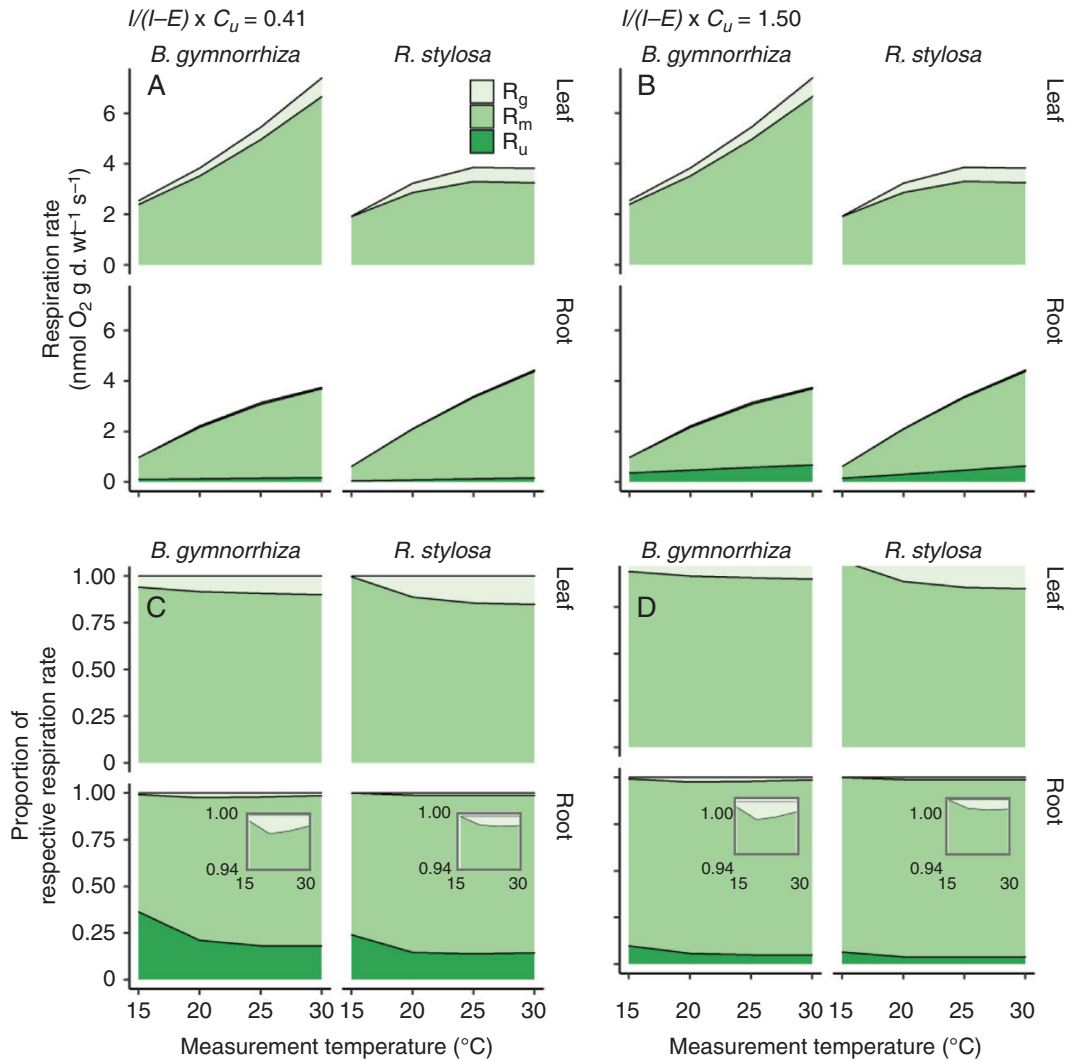


FIG. 8. O₂ respiration rates for growth (R_g), maintenance (R_m) and nitrogen uptake (R_u) at different growth temperatures in *B. gymnorhiza* and *R. stylosa*. In the R_u calculation, $\frac{I}{I-E} \times c_u$ was assumed to be 0.41 (A, C) or 1.50 (B, D). *I*, influx of nitrate into the cytoplasm; *E*, efflux (or leak) of nitrate out of the cytoplasm; *c_u*, specific cost of nitrate uptake. The upper parts show absolute values (A, B), and the lower parts show the proportions of R_g, R_m and R_u in total respiration rates (C, D). Enlarged graphs for roots are shown in (C) and (D). The temperatures on the x-axis are the measurement temperatures of O₂ respiration rates.

and *R. stylosa*, respectively, and 0.84 and 1.28 nmol O₂ g d. wt⁻¹ s⁻¹ in roots of *B. gymnorhiza* and *R. stylosa*) were not higher than O₂ respiration rates of temperate terrestrial plants (Zha *et al.*, 2001; van Iersel, 2006; Laureano *et al.*, 2008, 2013; Atkin *et al.*, 2015; Hernández-Montes *et al.*, 2019), suggesting that the distribution of the two mangroves in warm areas may not be caused by their high requirement for respiratory ATP at low growth temperatures. In the current study, at 15 °C growth temperature, the rates of N uptake were lower in *R. stylosa* roots than in *B. gymnorhiza* roots; this may be a reason for the lower growth rate of *R. stylosa* and its higher sensitivity to low temperature.

The leaf O₂ respiration rate of *B. gymnorhiza* tended to increase steadily with growth temperature (15–30 °C), whereas it peaked at 27.2 °C in *R. stylosa*. We have previously reported that rates of leaf CO₂ respiration and leaf assimilation of *R. stylosa* are saturated at 25 °C growth temperature (Akaji *et al.*, 2019). In the current study, the temperature of maximum leaf RGR was

higher in *B. gymnorhiza* (34.5 °C) than in *R. stylosa* (29.3 °C). These results suggest that the leaves of *B. gymnorhiza* are more evolutionarily adapted to high growth temperature than *R. stylosa* leaves. The slight increase in the CC and C content of *B. gymnorhiza* leaves at 30 °C growth temperature may also indicate better adaptation of this species. In some deciduous trees, an increase in the leaf content of lignin, which contains high amounts of C, helps to adjust to water stress under high light intensity (Niinemets and Kull, 1998).

Response of respiratory costs to growth temperatures in two mangrove species

Although we observed an increase in RGR with growth temperature in both species, large proportions of leaf and root O₂ respiration were used for maintenance at all growth temperatures examined (Fig. 8). The increase in O₂ respiration rates

as the growth temperature increased was due mainly to the increase in R_m . We found that specific rates of root O_2 respiration and NNUR were similar between *B. gymnorrhiza* and *R. stylosa* (Figs 1 and 6). Although specific rates of root ATP production and N uptake did not differ between the two species, *B. gymnorrhiza* had higher RMR and root RGR than *R. stylosa*. Thus, *B. gymnorrhiza* roots absorbed a lot of N at the whole-plant level, especially at above 20 °C growth temperature. The higher N uptake at the whole-plant level could be related to the higher Nc and leaf N content in *B. gymnorrhiza* than in *R. stylosa*. The higher leaf N content in *B. gymnorrhiza* indicates that the leaves of this species contained higher amounts of proteins to maintain; this could be one explanation for the high leaf R_m in *B. gymnorrhiza* at the high growth temperature. The leaf R_m and root R_m values of the two mangrove species were mostly within the range reported for temperate terrestrial plants, 2.0–15.6 nmol O_2 g d. wt⁻¹ s⁻¹ (Zha et al., 2001; van Iersel, 2006; Laureano et al., 2008; 2013; Hernández-Montes et al., 2019), and thus the two mangroves do not require high production of respiratory ATP for maintenance even at high growth temperatures.

In this study, we estimated the potential O_2 respiration rate for root N uptake, R_u , by using a theoretical equation. The assumed c_u and estimated R_u strongly depend on the nitrate efflux (E) and electron partitioning to the AP (τ_a) in roots [eqns (5) and (6)]. The ratio of E to nitrate influx (I) is 0.2–0.6 in other plant species (Bouma et al., 1996; Scheurwater et al., 1999; Ter Steege et al., 1999), and can differ among plant species (Scheurwater et al., 1999) and growth temperatures (Macduff et al., 1994). We used a constant for the ratio of E to I , but the ratio may change depending on the growth temperatures in the two mangrove species. Regarding electron partitioning to the AP (τ_a), the amounts of alternative oxidase (AOX) are influenced by growth temperature in various plant species (González-Meler et al., 1999; Kurimoto et al., 2004b; Fiorani et al., 2005; Watanabe et al., 2008; Umbach et al., 2009) and τ_a is affected by growth temperature in the leaves of *Populus canadensis* Moench (Searle and Turnbull, 2011) and *Quercus rubra* L. (Searle et al., 2011a). In many cases, electron partitioning to the AP is less than to the CP ($20/6 < P/O_2 < 29/6$) (Ribas-Carbo et al., 1995; Millar et al., 1998; Guy and Vanlerberghe, 2005; Armstrong et al., 2008; Macfarlane et al., 2009; Searle et al., 2011a, b). We assumed that c_u did not change with growth temperature or plant species in this study, but we cannot exclude that it does change. Based on the ratios of E to I and τ_a in previous studies, the actual R_u values of the two mangrove species may be in the range between the results of Fig. 8A and B. Further experiments to measure the nitrate efflux and τ_a dynamics of the mangroves at different growth temperatures will be needed to reveal the detailed R_u dynamics.

Conclusions

We analysed the responses of growth parameters to growth temperature in the context of respiration cost for maintenance and growth in seedlings of two mangrove species and found that both species require a threshold temperature (12.2 °C in *B. gymnorrhiza* and 18.1 °C in *R. stylosa*) to ensure an O_2 respiration rate sufficient for their leaf and root maintenance and

growth. The link between leaf and root growth parameters and O_2 respiration in the two mangrove species indicates that the underground temperature probably limits their growth under the low-temperature condition. Responses of whole-plant RGR to growth temperature showed that *B. gymnorrhiza* had the potential to adapt to a wider habitat temperature range than *R. stylosa*, which may be one explanation for the fact that *B. gymnorrhiza* grows in a wider range of western Indo-Pacific habitats than does *R. stylosa* (Spalding et al., 2010; Supplementary data Fig. S1). In native habitats, *B. gymnorrhiza* tends to be on inland waterways, and *R. stylosa* tends to grow by the seaside. *Bruguiera gymnorrhiza* often grow as juveniles in shaded environments underneath their mother tree canopies. Therefore, compared with *B. gymnorrhiza* seedlings, the seedlings of *R. stylosa* may be more exposed to strong irradiance with high temperatures in the daytime. This could also explain the narrow distribution range of *R. stylosa* on the global scale because the region in which the air temperature does not exceed the limiting temperature for the mangrove growth may be narrower at the seaside than inland. Our results suggest that the effects of global warming caused by climate change could differ between the two mangrove species. In the future, other factors controlling the warm habitat range of mangroves should be examined; for example, mangrove plants grow in tidal flats where salinity stress can affect respiratory cost for maintenance and growth. Some studies have shown that salinity stress can affect RGR and respiration rates in mangrove species, but the magnitudes of these effects vary greatly among studies, among species and among organs (Burchett et al., 1989; Lin and Sternberg, 1993; López-Hoffman et al., 2006; Atreya and Bharagava, 2008; Paliyavuth et al., 2009). Due to tidal export of organic matters from the soil, the levels of nutrients such as N and phosphorus (P) are low in tidal flats (Alongi et al., 1992). Lovelock et al. (2006) observed an increase in the fine root respiration rate of another mangrove species, *Rhizophora mangle* L., following extra input of N and P. Examination of respiratory costs at high salinity and low N and P will further reveal the acclimation and adaptation mechanisms of these mangrove species.

SUPPLEMENTARY DATA

Supplementary data are available online at <https://academic.oup.com/aob> and consist of the following. Table S1: F -values and P -values of two-way ANOVA of the effects of outside weather conditions and chamber on the chamber conditions. Table S2: F -values and P -values of one-way ANOVA of the effect of position on the daily accumulated air temperature, daily mean humidity and daily accumulated light intensity within a growth chamber. Table S3: F -values and P -values of two-way ANOVA for RGR, NAR, LAR, NNUR, LMR, RMR, LMA and Nc, and multifactor ANOVA for respiration rate, regression coefficient for growth, construction cost, protein content, C content, N content, nitrate N content and mineral content. Table S4: comparison of growth and respiration parameters in two mangrove species, *Bruguiera gymnorrhiza* and *Rhizophora stylosa* by Tukey's multiple comparison tests. Table S5: estimated parameters of the models of the growth temperature dependencies of the growth and respiration parameters of two mangrove species, *Bruguiera gymnorrhiza* and *Rhizophora stylosa*. Figure S1: global distribution map and estimated

range of air temperature of two mangrove species, *Bruguiera gymnorhiza* and *Rhizophora stylosa*. Figure S2: growth chambers used for the cultivation experiment. Figure S3: profiles of air temperature, humidity and light intensity in the growth chambers. Figure S4: relationship between air temperature in the growth chambers and solar radiation and outside air temperature. Figure S5: estimated air temperatures in the chambers during the 56 d cultivation period. Figure S6: relationship between temperatures at leaf positions and at depths of 10 cm in soils. Figure S7: contents of nitrate N and minerals in leaves and roots of *B. gymnorhiza* and *R. stylosa*.

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