



# Photosynthesis, respiration, and growth patterns of *Rhizophora stylosa* seedlings in relation to growth temperature

Yasuaki Akaji<sup>1</sup> · Tomomi Inoue<sup>1</sup> · Hajime Tomimatsu<sup>2</sup> · Ayumi Kawanishi<sup>1</sup>

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## Abstract

**Key message** *Rhizophora stylosa* Griff. exhibits thermal acclimatization of leaf respiration, but does not maintain high photosynthetic performance under new growth temperatures.

**Abstract** Mangrove plants are distributed across the majority of the world's tropical and subtropical coastlines. How they respond to new growth temperatures is poorly understood. To obtain this information, we collected *Rhizophora stylosa* Griff. diaspores from a subtropical region with average daily mean and maximum temperatures of 24.0 and 26.7 °C. After 1-year-old seedlings had been grown in four glasshouse chambers at an air temperature of 15, 20, 25, or 30 °C for about 50 days, we evaluated photosynthesis, respiration, and growth. Seedlings grown at 15 and 20 °C showed PSII photoinhibition, especially in newly emerged leaves. The assimilation rate was greatest at 25 °C. Thermal acclimatization of respiration was observed at 20–30 °C in both newly emerged and pre-existing leaves, was unclear in stems, and was absent in roots. Stem growth rate was greatest at 25 °C, but seedlings grown at 30 °C produced the largest number of new leaves and marginally decreased biomass allocation to roots, which lacked thermal acclimatization capacity. These results indicate that *R. stylosa* growing in subtropical regions adapts its photosynthesis to the average daily mean or maximum temperatures, and minimizes carbon loss due to warm temperatures by use of the thermal acclimatization capacity of leaf respiration and plasticity of biomass allocation, which contribute to optimizing growth performance.

**Keywords** Anthropogenic climate change · Biomass allocation · Chilling stress · Chlorophyll fluorescence · Leaf developmental stage

## Introduction

Plants synthesize carbohydrates through photosynthesis, and obtain the energy and carbon necessary for biosynthesis and cellular maintenance through respiration. These

physiological processes are strongly influenced by short-term variation in temperature. In general, photosynthesis increases with increases in temperature up to an optimum and decreases above that optimum, and respiration increases exponentially with increases in temperature (e.g., Yamori et al. 2005). However, if a plant is exposed to cold or warm temperatures for an extended period, unfavorable changes in photosynthesis and respiration might be mitigated by thermal acclimation processes (e.g., Berry and Björkman 1980; Atkin and Tjoelker 2003; Yamori et al. 2005; Way and Oren 2010; Slot and Kitajima 2015). However, if the capacities of photosynthesis and respiration to adjust were insufficient to compensate for a change in growth temperature, plant growth would decline. Therefore, to fully understand the growth patterns of plants growing under fluctuating temperatures and to predict the impact of anthropogenic climate change on plant performance and distribution, it is essential to assess how plants respond to altered growth temperatures.

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✉ Yasuaki Akaji  
yasuaki.a.1988@gmail.com

<sup>1</sup> Center for Environmental Biology and Ecosystem Studies, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan

<sup>2</sup> Center for Global Environmental Research, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan

Mangrove plants are distributed in an ecotone in the zone between sea and land in both tropical and subtropical regions. The total area of mangrove forests globally was estimated to be 152,360 km<sup>2</sup> (Spalding et al. 2010). These ecosystems contribute significantly to carbon storage (Donato et al. 2011), and provide timber products, nurseries for many fishes, and protection from coastal damage by intense storms (Spalding et al. 2010). Many researchers have suggested that minimum temperatures are the major factor limiting the performance and distribution of mangrove plants near their latitudinal limits (Woodroffe and Grindrod 1991; Stuart et al. 2007; Krauss et al. 2008; Quisthoudt et al. 2012; Osland et al. 2013, 2017a, b; Lovelock et al. 2016; Chen et al. 2017). Chen et al. (2017), for example, demonstrated that photosynthetic functions of most mangrove plants declined after an extreme cold temperature event in southern China. Ulqodry et al. (2014) showed that *Rhizophora mucronata* Lam., which originates in the tropics, had a lower photosynthetic rate when grown in a greenhouse in Japan in winter (approximate air temperature of 20 °C) than in summer (approximate air temperature of 30 °C), implying that mangrove plants are not able to acclimatize their photosynthesis to cooler temperatures. In contrast, it is still unclear whether they can maintain high photosynthetic performance when the temperature increases.

Unlike photosynthesis, thermal adjustment of respiration may occur rapidly (Ow et al. 2008a, b, 2010) through several processes such as changes in the regulation of existing enzymes, in the amounts of mitochondrial protein, and in the composition of individual enzymes (Atkin et al. 2005). However, the respiratory response of mangrove plants to new growth temperatures has not been assessed, although thermal adjustment of respiration has been widely examined in various organisms, including terrestrial plants (Way and Oren 2010; Slot and Kitajima 2015; Araki et al. 2017), freshwater macrophytes (Pilon and Santamaría 2001), and marine organisms such as seagrasses (Collier et al. 2017) and fishes (Robinson and Davison 2008; Sandblom et al. 2014, 2016). In addition to such physiological thermal adjustments, plants can change biomass allocation in response to growth temperature, though the outcomes are highly variable among species and environmental conditions (Callaway et al. 1994; Olszyk et al. 2003; Wang et al. 2013; Slot and Winter 2018). So far, there have been no experimental studies of the effect of growth temperature on biomass allocation in mangrove plants. Thus, an evaluation of the growth temperature responses of respiration, biomass allocation, and photosynthesis of mangrove plants should help us to understand how they cope with seasonal temperature fluctuations in the subtropics, and how they will respond to anthropogenic climate change. Our objective was, therefore, to elucidate how mangrove species growing in a subtropical region respond to different growth temperatures.

We focused on *Rhizophora stylosa* Griff., because it is a widespread mangrove species that is found from southern Australia to southern Japan (Tropical Coastal Ecosystems Portal 2018), and is a major component of mangrove forests in southern Japan (Kadoya and Inoue 2015). Its broad distribution might result from its ability to adjust its physiological functions in response to temperature fluctuations. We, therefore, hypothesized (1) that *R. stylosa* seedlings would exhibit strong thermal adjustment of respiration, and (2) that they would show differences in biomass allocation between different growth temperatures. We experimentally evaluated leaf photosynthesis, dark respiration of leaf, stem, and root, and growth patterns of seedlings grown at four different temperatures (15, 20, 25, and 30 °C) in glasshouse chambers in which air temperature and humidity can be controlled. Since physiological responses to new growth temperatures can differ between newly emerged and pre-existing leaves (Campbell et al. 2007; Ow et al. 2008a, b; Slot and Kitajima 2015), we quantified the photosynthesis and respiration responses in both.

## Materials and methods

### Experimental design

We collected diaspores of *R. stylosa* from more than 30 mature trees in a mangrove forest in Funaura Bay, Iriomote Island, Okinawa Prefecture, Japan (24°24'02"N, 123°48'33"E), on 5 July 2016. According to 2007–2016 meteorological data (JMA 2018), the average daily mean temperature on Iriomote Island was 24.0 °C, that in January (the coolest month) was 18.3 °C, and that in July (the warmest month) was 29.0 °C (Table S1). We planted the diaspores in trays filled with sand in a glasshouse (25 °C) and watered them twice daily. After about 3 months, we transplanted the seedlings individually into plastic pots (159 mm diameter; 246 mm depth). From that point, they were watered with 1:2000-diluted Hyponex<sup>®</sup> (Hyponex Japan, Osaka, Japan) twice daily until the end of the study.

We used four growth chambers to control temperature and humidity. The front, top, and sides of the chambers were made of glass. Since the growth chambers were inside a glasshouse, the seedlings in the chambers were under natural light filtered through two layers of glass. Each chamber was assigned to one air temperature treatment (15, 20, 25, or 30 °C), and the humidity of all chambers was set at 70%. On 9 August 2017, we selected 32 healthy seedlings and put 8 into each chamber. The means ( $\pm$ SD) of seedling heights on day 1 (i.e., when they were placed into the growth chambers) were 43.5 ( $\pm$ 3.6) cm at 15 °C, 42.8 ( $\pm$ 5.3) cm at 20 °C, 41.9 ( $\pm$ 5.1) cm at 25 °C, and 42.2 ( $\pm$ 4.1) cm at 30 °C.

## Measurements

For measurements of leaf gas exchange rate and chlorophyll fluorescence, we randomly selected four seedlings at each growth temperature. We measured both new leaves (leaves that had emerged and developed since day 1) and pre-existing leaves on each seedling from days 50 to 55 (28 September to 3 October 2017) in a portable photosynthesis system (LI-6400xt, LI-COR Inc., Lincoln, NE, USA) with a 2-cm<sup>2</sup> leaf chamber fluorometer (LI-6400-40, LI-COR Inc.). To adapt the seedlings to dark conditions, we covered them with light-shielding sheets the night before, and then measured dark respiration rates ( $R_{\text{dark}}$ , nmol g<sup>-1</sup> s<sup>-1</sup>). At the same time, we measured both initial fluorescence ( $F_o$ ) and maximum fluorescence just after a saturating flash ( $F_m$ ) under dark conditions and calculated maximum quantum yield of PSII photochemistry as  $(F_m - F_o)/F_m$  (i.e.,  $F_v/F_m$ ). The seedlings were then adapted to daylight for at least 1 h. We then measured gas exchange under light-saturated conditions (2000 μmol photons m<sup>-2</sup> s<sup>-1</sup>), stomatal water conductance ( $g_s$ , mol m<sup>-2</sup> s<sup>-1</sup>), transpiration rate ( $E$ , mmol m<sup>-2</sup> s<sup>-1</sup>), and light-saturated net assimilation rate ( $A_{\text{sat}}$ , μmol m<sup>-2</sup> s<sup>-1</sup>). We also measured the steady-state fluorescence ( $F_s$ ) and maximum fluorescence just after a saturating flash ( $F'_m$ ), and calculated the actual quantum yield of PSII photochemistry as  $(F'_m - F_s)/F'_m$  (i.e.,  $\Phi_{\text{PSII}}$ ) (Genty et al. 1989). These measurements were not performed on two of the new leaves of two seedlings (one at 15 °C and one at 20 °C), because the leaves were too small.

In addition, we measured dark respiration rates of main stems and roots of four selected seedlings at each growth temperature on days 56 and 57 (4 and 5 October 2017) with a portable photosynthesis system (LI-6400xt, LI-COR Inc.) with a 2-cm × 3-cm chamber (LI-6400-02B, LI-COR Inc.). We sampled part of the stem (upper part of hypocotyl) and roots (near the root tip) from each seedling that had been adapted to dark conditions as described above, and placed each sample in a mesh bag. Each mesh bag was then tightly enclosed in the 2-cm × 3-cm chamber, and measurements were conducted under dark conditions. The mean dry mass of stems in the mesh bags was 0.80 g, and that of roots was 0.12 g. To minimize wound-related respiration, we divided each stem sample into only two pieces and did not cut roots, and after the values stabilized, measured the respiration rates. During these measurements, the CO<sub>2</sub> concentration in the chamber was set to 400 ppm and the temperature was set to the treatment temperature.

We measured the height of the main stem, the diameter at the base of the main stem, and total branch length on days 1 and 51, and calculated the growth rate (mm day<sup>-1</sup>) of each. We also counted both newly emerged leaves and fallen leaves during the 51 days. After all measurements were taken, we measured leaf projected area (cm<sup>2</sup>) in LIA32

software (Yamamoto 2003) from digital images prepared with a scanner. We then dried the leaves, stems, and roots at 75 °C to a constant weight, weighed them, and calculated the leaf mass fraction (LMF), stem mass fraction (SMF), root mass fraction (RMF), and specific leaf area (SLA, cm<sup>2</sup> g<sup>-1</sup>) of both new and pre-existing leaves, and the leaf area ratio (LAR, cm<sup>2</sup> g<sup>-1</sup>) of each seedling. We excluded one seedling from the 15 °C treatment from the branch length calculation and from the number and biomass of new and pre-existing leaf calculations, because we failed to identify one branch of the seedling on day 1.

## Statistical analyses

We statistically tested the differences in physiological and growth variables between the four growth temperature treatments using Tukey's multiple comparison method, after assuming a normal, gamma, or Poisson distribution for each variable (Table S2). Statistical significance was accepted at  $P < 0.05$ . The analyses were performed using the "glht" function in the "multcomp" package (Hothorn et al. 2008) and the "glm" function of R v. 3.3.3 software (R Core Team 2017). For fluorescence, mass fractions, SLA, and LAR, the denominator was assigned to the offset term (Table S2). When dry mass of new leaves ( $g$ ) was 0, it was set to 0.001 and fitted to a gamma distribution.

## Calculation of thermal acclimatization index of respiration

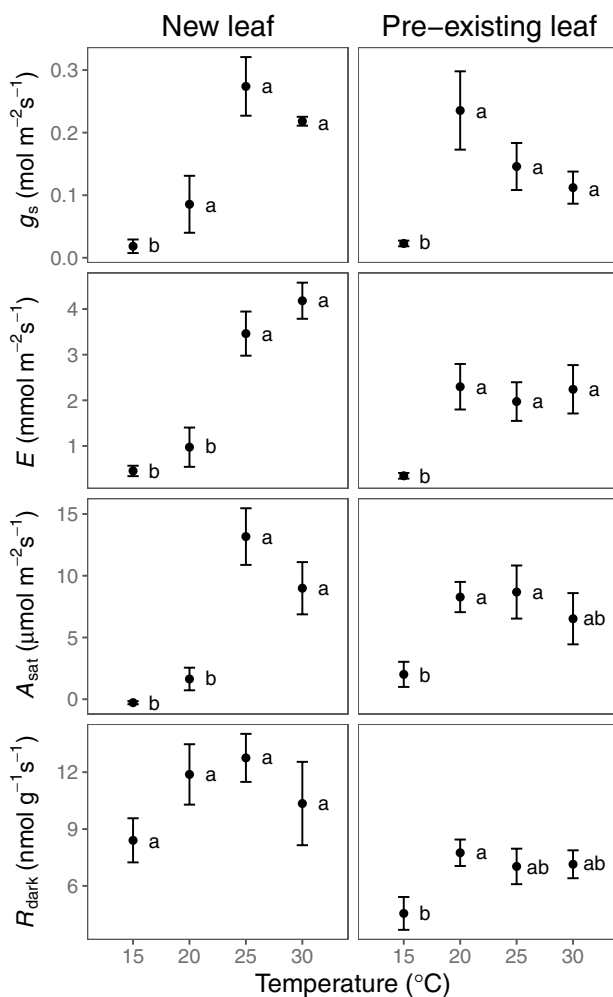
We additionally quantified the thermal respiratory acclimatization of new and pre-existing leaves, stems, and roots using the homeostasis method (i.e.,  $\text{Acclim}_{\text{Homeo}}$  index) as described in Loveys et al. (2003) (see also Slot and Kitajima 2015).  $\text{Acclim}_{\text{Homeo}}$  is the ratio of respiration of plants grown and measured at a cooler temperature to plants grown and measured at a warmer temperature. We calculated mean  $R_{\text{dark}}$  at 15 °C/mean  $R_{\text{dark}}$  at 25 °C, mean  $R_{\text{dark}}$  at 20 °C/mean  $R_{\text{dark}}$  at 25 °C, and mean  $R_{\text{dark}}$  at 25 °C/mean  $R_{\text{dark}}$  at 30 °C. According to Slot and Kitajima (2015), respiratory homeostasis is complete when  $\text{Acclim}_{\text{Homeo}} = 1.0$ , incomplete when  $\text{Acclim}_{\text{Homeo}} < 1.0$ , and overcompensating when  $\text{Acclim}_{\text{Homeo}} > 1.0$ .

## Results

### Leaf gas exchange and chlorophyll fluorescence

In newly emerged leaves, mean stomatal conductance ( $g_s$ ) increased as growth temperature increased from 15 °C and became saturated at 25 °C, and mean transpiration rate ( $E$ ) increased linearly from 15 to 30 °C;  $g_s$  and  $E$  did not differ

significantly between 25 and 30 °C (Fig. 1). Mean assimilation rates ( $A_{\text{sat}}$ ) of the seedlings grown at 15 and 20 °C were much lower than those at 25 and 30 °C, while that of the seedlings grown at 25 °C was marginally higher than that at 30 °C;  $A_{\text{sat}}$  was optimal at 25 °C (Fig. 1). Mean dark respiration rates ( $R_{\text{dark}}$ ) of the seedlings grown at 20 and 25 °C were higher than those at 15 and 30 °C, resulting in an apparent bell curve against growth temperature, but there were no significant differences between temperatures (Fig. 1). In pre-existing leaves, the means of the four gas exchange parameters were lowest in seedlings grown at 15 °C, and the mean  $g_s$  of the seedlings grown at 20 °C was marginally higher than those at 25 and 30 °C (Fig. 1).



**Fig. 1** Stomatal conductance ( $g_s$ ), transpiration rate ( $E$ ), light-saturated net assimilation rate ( $A_{\text{sat}}$ ), and dark respiration rate ( $R_{\text{dark}}$ ) of newly emerged and pre-existing leaves of *Rhizophora stylosa* seedlings ( $n=3$  or 4) from days 50 to 55 in the growth chambers at each growth temperature. Points and bars indicate mean and SEM of each treatment. Values with the same letter are not significantly different between temperatures by Tukey's multiple comparison test ( $P < 0.05$ )

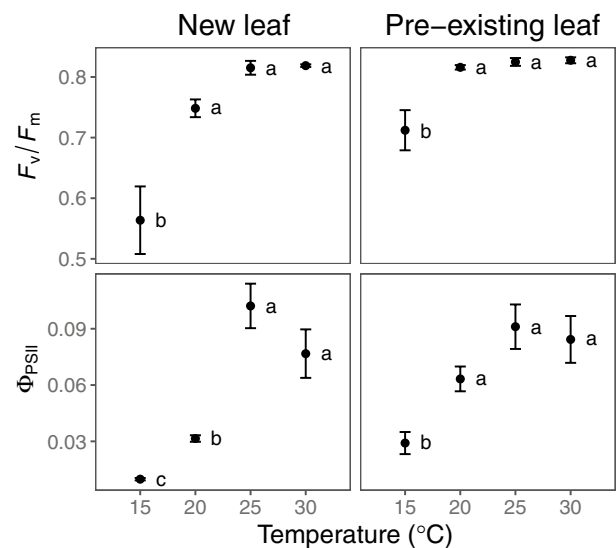
The mean maximum quantum yield of PSII ( $F_v/F_m$ ) of new leaves of the seedlings grown at 15 and 20 °C and that of pre-existing leaves grown at 15 °C was  $< 0.8$  (Fig. 2), indicating a decline in PSII function. In particular, that of new leaves of the seedlings grown at 15 °C was lowest, at 0.56. The actual quantum yield of PSII ( $\Phi_{\text{PSII}}$ ) showed a similar trend to that of the assimilation rate (Figs. 1, 2).

### Stem and root respiration

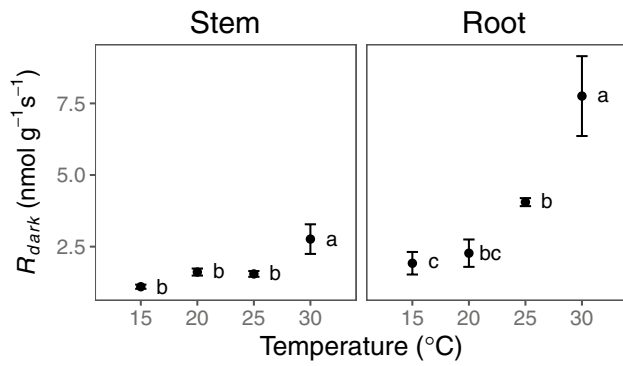
The mean  $R_{\text{dark}}$  values of main stems and roots were highest at 30 °C and were low at the other temperatures (Fig. 3). That of roots appeared to increase exponentially with growth temperature.

### Thermal acclimatization of respiration

Acclim<sub>Homeo</sub> between 15 and 25 °C was 0.66 in newly emerged leaves and 0.65 in pre-existing leaves (Table 1), indicating incomplete homeostasis of leaf respiration. That between 20 and 25 °C was 0.93 in newly emerged leaves and 1.10 in pre-existing leaves (Table 1), indicating slightly incomplete homeostasis of respiration of newly emerged leaves and slight overcompensation in pre-existing leaves. That between 25 and 30 °C was 1.23 in newly emerged leaves and 0.98 in pre-existing leaves (Table 1), indicating that homeostasis of respiration of newly emerged leaves was higher than that of pre-existing leaves.



**Fig. 2** Maximum ( $F_v/F_m$ ) and actual quantum yields ( $\Phi_{\text{PSII}}$ ) of PSII of newly emerged and pre-existing leaves of *Rhizophora stylosa* seedlings ( $n=3$  or 4) from days 50 to 55 in the growth chambers at each growth temperature. Points and bars indicate mean and SEM of each treatment. Values with the same letter are not significantly different between temperatures by Tukey's multiple comparison test ( $P < 0.05$ )



**Fig. 3** Dark respiration rates ( $R_{\text{dark}}$ ) of main stem and roots of *Rhizophora stylosa* seedlings ( $n=4$ ) on days 56 and 57 in the growth chambers at each growth temperature. Points and bars indicate mean and SEM of each treatment. Values with the same letter are not significantly different between temperatures by Tukey’s multiple comparison test ( $P < 0.05$ )

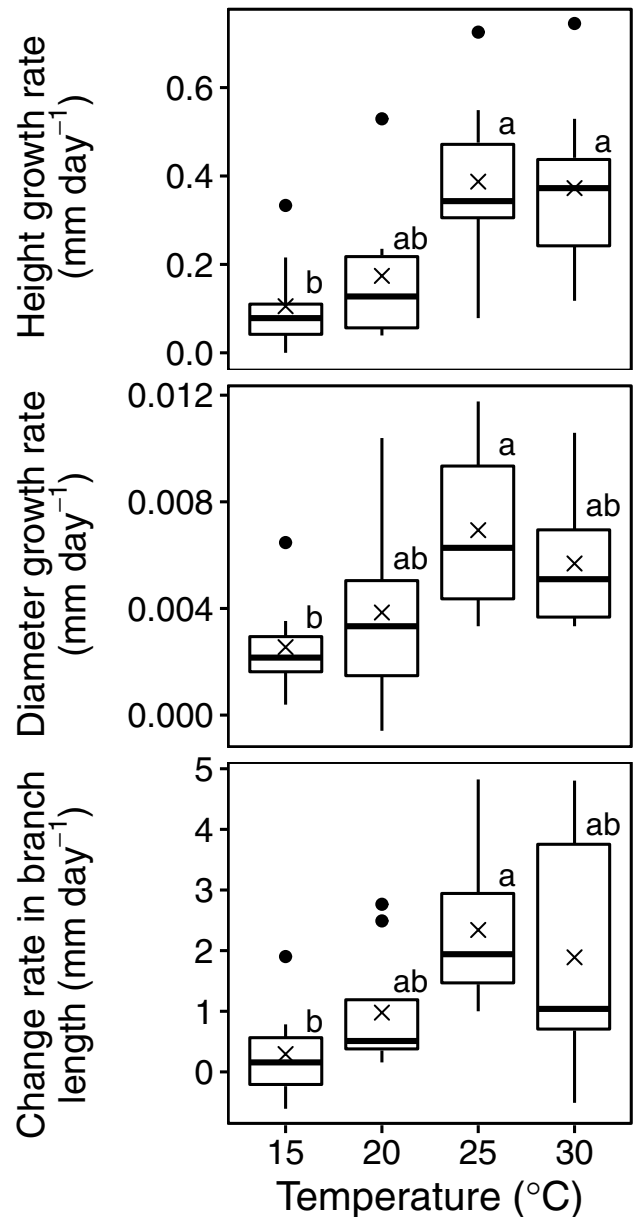
**Table 1** Degrees of homeostasis of respiration ( $\text{Acclim}_{\text{Homeo}}$ ) of newly emerged leaves, pre-existing leaves, stems, and roots of *Rhizophora stylosa* seedlings

Comparison	Leaf		Stem	Root
	New	Pre-existing		
Mean $R_{\text{dark}}$ at 15 °C/mean $R_{\text{dark}}$ at 25 °C	0.66	0.65	0.71	0.47
Mean $R_{\text{dark}}$ at 20 °C/mean $R_{\text{dark}}$ at 25 °C	0.93	1.10	1.04	0.56
Mean $R_{\text{dark}}$ at 25 °C/mean $R_{\text{dark}}$ at 30 °C	1.23	0.98	0.56	0.52

$\text{Acclim}_{\text{Homeo}}$  between 15 and 25 °C was 0.71 in stems and 0.47 in roots (Table 1), indicating incomplete homeostasis. That between 20 and 25 °C was 1.04 in stems and 0.56 in roots (Table 1), indicating complete homeostasis of respiration in stems and incomplete homeostasis in roots. That between 25 and 30 °C was 0.56 in stems and 0.52 in roots (Table 1), indicating incomplete homeostasis.

**Stem growth rate, leaf dynamics, and biomass allocation**

All seedlings survived in all four growth chambers. The mean growth rates of the height of the main stem, the diameter at the base of the main stem, and total branch length were highest at 25 °C, followed in order by 30, 20, and 15 °C; differences between 15 and 25 °C were significant (Fig. 4). The means of the total number and dry mass of newly emerged leaves increased linearly with growth temperature from 15 to 30 °C, though dry mass differed significantly only between 15 °C and the three higher temperatures (Table 2). The mean of the total number of dead leaves increased as growth



**Fig. 4** Boxplots showing the rate of growth of trunk height and diameter and the rate of change of branch length of *Rhizophora stylosa* seedlings ( $n=7$  or 8) over 51 days at each growth temperature. Crosses represent means. Values with the same letters are not significantly different between temperatures by Tukey’s multiple comparison test ( $P < 0.05$ )

temperature increased from 15 to 25 °C and stayed almost the same at 30 °C, and significant differences were detected between 15–20 °C and 25–30 °C (Table 2). The means and variations of dry masses of pre-existing leaves, stems, and roots were highest at 20 °C, and the mean root dry mass was lowest at 30 °C, though there were no significant differences between the four temperatures (Table 2). There were no significant differences in LMF, SMF, RMF, SLA of new or pre-existing leaves, or LAR among the four temperatures

**Table 2** Means ( $\pm$ SD) of numbers of new and dead leaves, dry mass, mass fraction, SLA, and LAR of *Rhizophora stylosa* seedlings at each growth temperature treatment

Variable	Growth temperature ( $^{\circ}$ C)			
	15	20	24	30
Total number of new leaves in the 51 days	4.6 ( $\pm$ 5.4) <sup>c</sup>	10.5 ( $\pm$ 7.5) <sup>b</sup>	13.8 ( $\pm$ 5.4) <sup>ab</sup>	17.5 ( $\pm$ 8.3) <sup>a</sup>
Total number of dead leaves in the 51 days	2.7 ( $\pm$ 3.8) <sup>b</sup>	4.0 ( $\pm$ 2.6) <sup>b</sup>	7.4 ( $\pm$ 3.9) <sup>a</sup>	7.3 ( $\pm$ 3.5) <sup>a</sup>
Dry mass of new leaves (g)	0.5 ( $\pm$ 0.8) <sup>b</sup>	1.7 ( $\pm$ 1.4) <sup>a</sup>	2.6 ( $\pm$ 1.1) <sup>a</sup>	2.8 ( $\pm$ 1.4) <sup>a</sup>
Dry mass of pre-existing leaves (g)	8.7 ( $\pm$ 1.2) <sup>a</sup>	10.7 ( $\pm$ 3.8) <sup>a</sup>	9.2 ( $\pm$ 2.3) <sup>a</sup>	8.6 ( $\pm$ 1.9) <sup>a</sup>
Dry mass of stems (g)	21.0 ( $\pm$ 3.5) <sup>a</sup>	23.0 ( $\pm$ 7.4) <sup>a</sup>	21.6 ( $\pm$ 3.3) <sup>a</sup>	21.4 ( $\pm$ 3.8) <sup>a</sup>
Dry mass of roots (g)	4.7 ( $\pm$ 1.1) <sup>a</sup>	5.2 ( $\pm$ 1.6) <sup>a</sup>	4.8 ( $\pm$ 1.3) <sup>a</sup>	4.2 ( $\pm$ 0.8) <sup>a</sup>
LMF	0.28 ( $\pm$ 0.05) <sup>a</sup>	0.30 ( $\pm$ 0.04) <sup>a</sup>	0.31 ( $\pm$ 0.04) <sup>a</sup>	0.31 ( $\pm$ 0.05) <sup>a</sup>
SMF	0.59 ( $\pm$ 0.03) <sup>a</sup>	0.57 ( $\pm$ 0.03) <sup>a</sup>	0.57 ( $\pm$ 0.03) <sup>a</sup>	0.58 ( $\pm$ 0.05) <sup>a</sup>
RMF	0.13 ( $\pm$ 0.03) <sup>a</sup>	0.13 ( $\pm$ 0.01) <sup>a</sup>	0.12 ( $\pm$ 0.02) <sup>a</sup>	0.11 ( $\pm$ 0.01) <sup>a</sup>
SLA of new leaves ( $\text{cm}^2 \text{g}^{-1}$ )	68.4 ( $\pm$ 16.7) <sup>a</sup>	76.2 ( $\pm$ 7.8) <sup>a</sup>	68.8 ( $\pm$ 2.7) <sup>a</sup>	73.5 ( $\pm$ 4.0) <sup>a</sup>
SLA of pre-existing leaves ( $\text{cm}^2 \text{g}^{-1}$ )	52.3 ( $\pm$ 5.5) <sup>a</sup>	49.7 ( $\pm$ 2.5) <sup>a</sup>	48.7 ( $\pm$ 4.4) <sup>a</sup>	50.2 ( $\pm$ 3.0) <sup>a</sup>
LAR ( $\text{cm}^2 \text{g}^{-1}$ )	14.5 ( $\pm$ 3.5) <sup>a</sup>	16.2 ( $\pm$ 2.3) <sup>a</sup>	16.4 ( $\pm$ 2.1) <sup>a</sup>	17.4 ( $\pm$ 2.8) <sup>a</sup>

Numbers followed by the same letter are not significantly different between temperatures by Tukey's multiple comparison test ( $P < 0.05$ ,  $n = 5-8$ )

LMF leaf mass fraction, SMF stem mass fraction, RMF root mass fraction, SLA specific leaf area, LAR leaf area ratio

(Table 2). However, LMF and LAR tended to increase with temperature from 15 to 30  $^{\circ}$ C, and RMF tended to decrease.

## Discussion

### Photosynthetic response of *R. stylosa* seedlings to different growth temperatures

Optimum temperatures for photosynthetic rate of three mangrove species (*Avicennia marina* (Forssk.) Vierh., *Bruguiera gymnorhiza* (L.) Lam., and *Rhizophora apiculata* Blume) were between 25 and 30  $^{\circ}$ C, approximately the average air temperature in the field (Ball et al. 1988). The photosynthetic rates of 42 non-mangrove tropical tree and liana species were optimal at the average maximum temperature in the field (Slot and Winter 2017a). We found that  $A_{\text{sat}}$  of *R. stylosa* seedlings was optimal at 25  $^{\circ}$ C (Fig. 1), close to the average daily mean (24.0  $^{\circ}$ C) and maximum (26.7  $^{\circ}$ C) temperatures on Iriomote Island, where the diaspores were collected (Table S1). Accordingly, our findings support the previous results that photosynthesis of tropical trees is adapted to the average daily mean or maximum temperature, and suggest that *R. stylosa* cannot maintain a high photosynthetic performance when the growth temperature changes, despite a 50-day acclimatization period. Partial acclimatization of photosynthesis can be supported by the results of *R. mucronata* (Ulqodry et al. 2014), of *Avicennia germinans* (L.) L., a cold-tolerant mangrove species (Reef et al. 2016), and of three non-mangrove tropical tree species (Slot and Winter 2017b), and by incomplete changes in optimum temperature for non-tropical plants (Yamori et al. 2014).

The previous field work revealed that  $F_v/F_m$  of *R. stylosa* drastically declined after cold weather in southern China (Chen et al. 2017). We also found PSII photoinhibition in *R. stylosa* seedlings grown at 15  $^{\circ}$ C, lower than the average daily minimum temperature of the coolest month (16.1  $^{\circ}$ C) on Iriomote (Table S1), in both new and pre-existing leaves, and very low  $A_{\text{sat}}$  (Figs. 1, 2). Moreover, newly emerged leaves of seedlings grown at 20  $^{\circ}$ C showed PSII photoinhibition and low  $A_{\text{sat}}$  (Figs. 1, 2), although the temperature was higher than the average daily minimum and mean temperatures of the coolest month on Iriomote. The higher sensitivity of newly emerged leaves to low temperatures is consistent with the previous work using poplar saplings (Ow et al. 2008a). We, therefore, suggest that leaves of *R. stylosa* in the field can experience cold temperature stresses in winter.

$A_{\text{sat}}$  of the seedlings grown at 30  $^{\circ}$ C was slightly lower than that at 25  $^{\circ}$ C (Fig. 1). As the average daily mean and maximum temperatures in the warmest month were 29.0 and 32.1  $^{\circ}$ C, respectively, on Iriomote (Table S1), the 30  $^{\circ}$ C growth temperature is almost identical to the air temperature in the field during the warmest month. Therefore, *R. stylosa* may show slightly lower photosynthetic performance in the field during the warmest month of the year than during the intermediate period between the warmest and coldest months. The decline in  $A_{\text{sat}}$  of the seedlings grown at 30  $^{\circ}$ C could be due to stomatal closure to prevent leaf water deficits (Fig. 1). Mangrove trees are likely to close their stomata at high temperatures (Ball 1988); indeed, stomatal conductance of *R. stylosa* decreased from about 28  $^{\circ}$ C to about 40  $^{\circ}$ C (Andrews and Muller 1985).

## Respiratory response of *R. stylosa* to different growth temperatures

The previous studies demonstrated that thermal acclimatization of respiration of non-mangrove species occurred rapidly (Ow et al. 2008a, b, 2010). We observed that thermal acclimatization of leaf respiration of *R. stylosa* occurred between 20 and 30 °C (Table 1; Fig. 1), partly supporting our hypothesis of thermal adjustment. Thus, *R. stylosa* could not sufficiently acclimatize its leaf respiration when exposed to a temperature below the normal average daily minimum or mean temperature. Our finding that leaf respiration was downregulated at high growth temperature also supports the results of previous studies (Pilon and Santamaría 2001; Ow et al. 2008a, b, 2010; Way and Oren 2010; Slot and Kitajima 2015; Araki et al. 2017). Furthermore, we found that  $\text{Acclim}_{\text{Homeo}}$  between 25 and 30 °C in new leaves of *R. stylosa* was 1.23 (Table 1), higher than that of most non-mangrove plant species described in Slot and Kitajima (2015). This result suggests that *R. stylosa* produced highly acclimatized leaves to reduce the carbon loss associated with cellular maintenance.  $\text{Acclim}_{\text{Homeo}}$  between 25 and 30 °C in pre-existing leaves of *R. stylosa* was 0.98 (Table 1), indicating that those leaves were able to completely acclimatize their respiration to warming, but that the degree of downregulation of pre-existing leaves was not as great as that of newly emerged leaves. The higher acclimatization capacity of new leaves to warming has also been shown in other woody plants (Ow et al. 2008a, b; Slot and Kitajima 2015).

It was unclear whether stem respiration showed thermal acclimatization, but root respiration did not (Table 1; Fig. 3). This finding supports the hypothesis suggested by Slot and Winter (2018) that stem and root respiration of tropical species has a lower thermal acclimatization capacity than that of leaves, possibly because leaves are subjected to larger temperature fluctuations; in particular, mangrove roots would be protected from large fluctuations, even near the latitudinal limit for mangroves, because the seawater temperature range is narrower than that of air temperature (Wang et al. 2011; Quisthoudt et al. 2012). This assumption could be supported by the finding of no thermal adjustment of respiration in two tropical seagrass species, *Halodule uninervis* and *Cymodocea serrulata*, in Moreton Bay, Australia (Collier et al. 2017). These results prompt us to further speculate that stems of a mangrove plant might experience weaker temperature fluctuations than leaves, being mostly shaded from direct sunlight, but stronger fluctuations than roots, and that these differences might lead to the observed differences in thermal acclimatization capacity between leaf, stem, and root. However, the respiration rates of stem and roots that we measured might be somewhat increased by wound-related respiration, and we used only small samples

for measurements. Further studies are, therefore, needed to clarify this hypothesis.

## Growth pattern of *R. stylosa* at different growth temperatures

Stem growth rate was highest at 25 °C (Fig. 4). The seedlings grown at 30 °C produced the largest leaf number and new leaf biomass and tended to decrease their biomass allocation to roots, leading to the highest LAR value (Table 2). These results support our second hypothesis that *R. stylosa* will produce different biomass allocation patterns depending on the growth temperature. As a result, carbon loss of the seedlings grown at 30 °C might be suppressed by the increase in leaf biomass and decrease in root biomass, because respiration was downregulated in leaves but not in roots (Figs. 1, 3), possibly at the expense of water and nutrient uptake functions. Plasticity and patterns of biomass allocation in relation to warming vary among studies and species (Olszyk et al. 2003; Zhao and Liu 2009; Wang et al. 2013; Slot and Winter 2018); our results are consistent with that of the night-warming experiment conducted using a non-mangrove tropical tree species described by Slot and Winter (2018). Thus, the differences in growth pattern observed would be reduced if nighttime temperature were lower than daytime temperature, not equal as in our study. In addition, the means and variations of dry masses of pre-existing leaves, stems, and roots were greatest at 20 °C (Table 2), but we assume that this trend was due mainly to the presence of the largest (or heaviest) seedling in the 20 °C treatment (data not shown). Accordingly, we have avoided discussing this trend in terms of the growth temperature effect.

## Conclusions

We suggest that *R. stylosa* growing in subtropical regions adapts its photosynthesis to the average daily mean or maximum temperature in the field, and that thermal acclimatization capacity and biomass allocation plasticity can contribute to the maintenance of the seedlings' carbon balance when growth temperature increases. Moreover, we expect that a moderate warming effect from anthropogenic climate change will have a positive effect on the overall growth of *R. stylosa* in subtropical regions, because the degree of decline in photosynthetic performance was larger when the growth temperature decreased than when it increased, in line with predictions for mangrove expansion in east Asia (Osland et al. 2017b). Therefore, our results could be useful for predicting the changes in performance and distribution of *R. stylosa*. Further similar studies using several other mangrove species would provide additional insights to enable the prediction of changes in the composition of mangrove communities and

in climate-terrestrial carbon feedback under the effects of anthropogenic climate change.

**Author contribution statement** TI conceived the experiment. YA, TI, HT, and AK conducted the measurements. YA analyzed the data and wrote the paper. YA, TI, and HT revised the paper.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflicts of interest.

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