

Gas Exchange Analysis for Estimating Net CO₂ Fixation Capacity of Mangrove (*Rhizophora stylosa*) Forest in the Mouth of River Fukido, Ishigaki Island, Japan

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Abstract : Mangrove trees have been considered to possess a higher carbon fixation capacity than terrestrial trees although a reliable method to estimate their CO₂ fixation capacity has not been established. In this study, net CO₂ fixation in above-ground of *Rhizophora stylosa* was estimated as the difference between photosynthetic absorption and respiratory emission of CO₂. In order to estimate these parameters, photosynthetic rates of single-leaves in response to light and temperature and the respiratory rates of leaves and branches in response to temperature were measured. Furthermore, we established a model of diurnal change in temperature. Monthly averages of the diurnal temperature change were used for correcting the CO₂ absorption and emission. The effect of temperature modification on the estimation of net CO₂ fixation was examined, and the net CO₂ fixation capacity estimated with and without temperature modification was compared. Biomass accumulation estimated without temperature modification (i.e. corrected only for the light intensity) was 6.1 tons ha⁻¹ yr⁻¹, while that estimated with temperature modification (i.e. corrected for both light intensity and temperature) was 13.0 tons ha⁻¹ yr⁻¹. A doubling of the estimated values of net CO₂ fixation as observed in this study was caused by the decrease in respiratory CO₂ emission by half, which results from temperature modification. These findings suggest that temperature modification in gas exchange analysis could improve the accuracy of estimation of the net CO₂ fixation capacity.

Key words : CO₂ fixation, Gas exchange, Mangrove, Photosynthesis, Respiration, Temperature modification.

In recent decades, mangroves have been planted to counter the effect of degradation by human activities in coastal ecosystems of tropics and subtropics. The objective of the reforestation is not only a restoration, but also an expansion of the forest area, because forests are widely regarded as a significant sink for atmospheric CO₂ (Clough, 1998). Woody plants can accumulate much more CO₂ than herbages in the long term. This idea has been used as one of the countermeasures to reduce atmospheric CO₂ which was considered as the cause of global-warming in the Kyoto Protocol (1997).

Rhizophora stylosa (Japanese name, Yaeyamahirugi) is the mangrove species commonly distributed in the subtropical region of South-East Asia. The mangrove has been considered as an important local sink for atmospheric CO₂ along tropical coastlines (Ong, 1993). Komiyama (2004) reported that above-ground biomass of mangrove forests is comparable with that of terrestrial forests in the temperate zone. Komiyama (2004) also noted that 1,000 to 2,000 tC ha⁻¹ of carbon is stocked below-ground in mature *Rhizophora* forests. Thus, mangrove trees have been considered widely

to possess a higher carbon fixation capacity than terrestrial trees. The higher carbon fixation capacity of mangrove trees shows possibilities for their use in Clean Development Mechanism (CDM) programs of the Kyoto Protocol. However, a reliable method to estimate CO₂ fixation capacity of mangrove forests has not been established.

The CO₂ fixation capacity of mangrove trees has been estimated mainly by the allometric method based on the correlation between the trunk diameter at breast height (DBH, i.e. 1.3 m) and the single stand biomass (Ong et al., 1984, 1995; Putz and Chan, 1986; Clough and Scott, 1989; Ong, 1993; Matsui, 1998; Komiyama et al., 2005). This measurement is painstakingly hard and time-consuming (Clough et al., 1997), taking up to 1 to 5 years to establish the relationship between the trunk diameter and plant biomass. Thus, this method is not suitable for a large-scale survey and comparative studies involving different species in different areas and climates.

Gas exchange analysis is an alternate method based on estimation of CO₂ absorption as a gross productivity of the canopy. This is obtained by measuring leaf

photosynthetic rates, leaf area index (LAI), and light extinctions in the canopy (Bunt et al., 1979; Boto et al., 1984; Ong et al., 1995; Clough et al., 1997; Clough, 1998). Net canopy productivity can be estimated as the difference between the photosynthetic absorption and the respiratory emission of CO₂, although canopy respiration has scarcely been estimated. Ong et al. (1995) estimated the respiratory CO₂ emission of 20-year-old *Rhizophora apiculata* in Malaysia using the gas exchange method. However, they substituted the respiration rate of single leaves for the respiration rate of all above-ground organs, and the respiratory CO₂ emission has never been estimated adequately for either above- and below-ground organs (Gong and Ong, 1990; Clough et al., 1997). This is partly due to technical difficulties in measuring the respiration rate of non-assimilation organs and collecting root tissues (Clough, 1998).

An effective, direct and convenient way of estimating CO₂ fixation is the use of the gas exchange analysis method. This method is presumed better and easier than the allometric method. It enables estimation of CO₂ fixation capacity of stand or canopy by integrating CO₂ assimilation of each organ under variable meteorological conditions. This is carried out by measuring the gas exchange responses to environmental factors. However, although light intensity has been taken into account, little quantitative information is available on the effect of temperature on net primary production and growth (Clough, 1992). The previous studies using the gas exchange method did not take into account the temperature effect in the estimation (Bunt et al., 1979; Boto et al., 1984; Ong et al., 1995; Clough et al., 1997; Clough, 1998). This was because they used an instantaneous rate of net photosynthesis in the calculation, which was obtained by a former-type portable photosynthetic measurement system such as LI-6200 (Li-Cor, USA). This enabled data collection only under ambient conditions in their research period. Hence, these data were not sufficient to estimate the mangrove productivity under annual temperature variations. Clough (1998) estimated mangrove productivity by the traditional gas exchange method using the light response function obtained by Cheeseman et al. (1991), although the latter partly reported that the relationship between the net photosynthetic rate of leaf and leaf temperature indicated a broad peak between 29 and 34°C. Moore et al. (1973) reported the effect of temperature on the photosynthetic rate of *Rhizophora*, *Avicennia*, and *Laguncularia* species showing that the maximum photosynthetic rate was obtained at a leaf temperature near or below 25°C.

In this study, which was aimed at establishing the gas exchange analysis method, net CO₂ fixation in the above-ground organs of *R. stylosa* was estimated as the difference between photosynthetic absorption

and respiratory emission of CO₂. We added temperature modification to increase the accuracy of the estimation on net CO₂ fixation, which was corrected for light intensity and calculated at the annual average temperature of 25°C. The temperature modification was conducted using the temperature fluctuation model established during the day and night. The CO₂ fixation estimated with and without the temperature modification was compared. *R. stylosa* stands studied are located in the northern limits of mangrove distribution in the world, and the data gives information on mangrove productivity in the northern limits. The vegetative data of *R. stylosa* trees such as forest structure may also provide additional information for the estimation of mangrove productivity by another method such as remote-sensing.

Materials and Methods

1. Study site

Ishigaki Island is located at 24° 30' N and 124° 25' E in the East China Sea. Mature stands of mangroves growing in the mouth of Fukido River are some of the rare wild mangrove forests designated as a national monument in Japan. The dominant species in this area is *Rhizophora stylosa*. *Bruguiera gymnorrhiza* (Japanese name, Ohirugi) grows sparsely in this area. This region is characterized by a subtropical type of climate. The annual mean temperature ranges between 25.8°C at maximum and 21.8°C at minimum, the average humidity is 76%, and the annual precipitation is 2188 mm (period 1979-2000).

This study was carried out in September 2000 and December 2001. The stands investigated in this study (approximate tree diameter at breast height (DBH) and tree height were 8.3 cm and 5.5 m, respectively) were growing inside the forest, 50 m away from the edge of the river. The density was 54.0 stands / 100 m² (Matsui, 2000).

2. Estimation of a net CO₂ fixation capacity using gas exchange analysis

Net CO₂ fixation capacity in the above-ground organs of *R. stylosa* was calculated as the difference between photosynthetic absorption and respiratory emission of CO₂. CO₂ absorption of the canopy was calculated by integrating the photosynthetic CO₂ exchange rate (PCER) corrected for light intensity and temperature, and leaf distribution and the light extinction in the canopy. The CO₂ emission from each organ was calculated by multiplying the respiratory CO₂ emission rate (RCER) corrected for temperature of each above-ground organ such as trunk and branch by total biomass of the each above-ground organ. The absorption and emission of CO₂ per day was determined from the monthly average and were corrected for diurnal fluctuations of light intensity

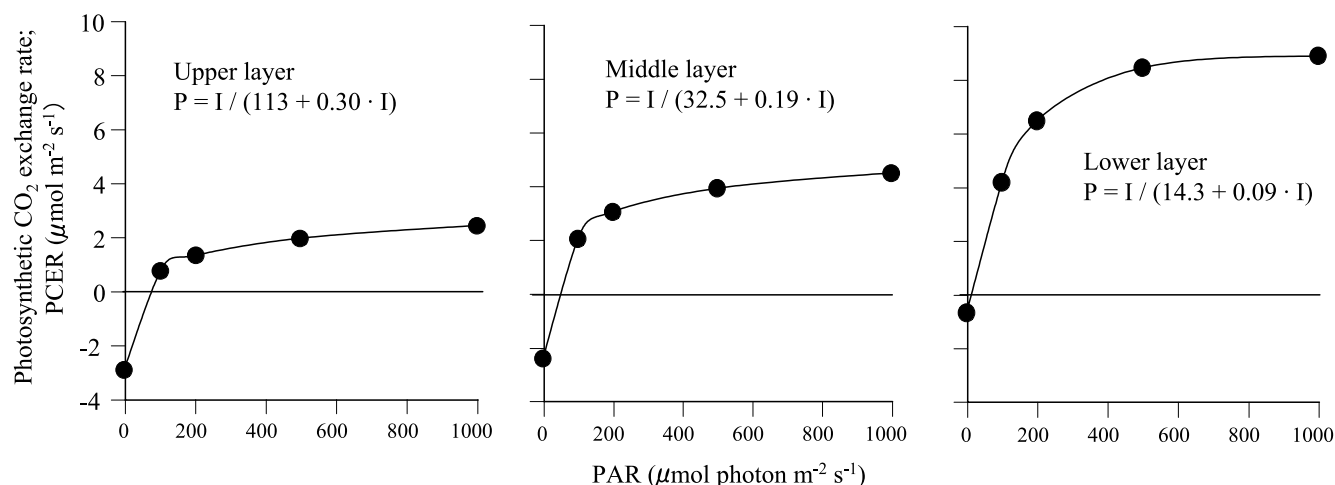


Fig. 1. Influence of light intensity on photosynthetic CO₂ exchange rate (PCER) measured in the leaves of *Rhizophora stylosa* located in three layers of the canopy. They were measured at leaf temperature 25°C. PAR in X-axis shows photosynthetically active radiation.

and temperature. An annual CO₂ fixation capacity was estimated by integrating the monthly difference between the absorption and emission of CO₂.

3. Leaf photosynthesis in the canopy in relation to light intensity

Steel towers were constructed (ca. 6 m in height, depending on the height of canopy) to measure photosynthetic traits of leaves located at the top of the canopy.

The PCER in fully expanded leaves located in upper, middle, and lower layers of the canopy was measured in September, 2000. This was done using light supplied by a portable photosynthesis measuring system (LI-6400, Li-Cor, USA). The intensity of photosynthetically active radiation (PAR) on leaf surfaces was automatically controlled in decreasing order from 1000 to 0 μmol m⁻² s⁻¹. During the measurements, leaf temperature was maintained at 25°C, vapor pressure deficit between the leaf and air (VpdL) was 1.7±0.2 kPa, and CO₂ concentration of the reference air was 370 μmol mol⁻¹.

Data collection was finished before 11 o'clock because the PCER gradually decreased. Such decrease may be caused by artificial stimuli from attaching the LI-6400 chamber to leaf. In fact, instantaneous measurement of mangrove photosynthesis measured using LI-6200 (Li-Cor) did not decrease to zero (there might have midday depression) (eg. Cheeseman et al., 1991). The decrease of PCER was not utilized to estimate net CO₂ fixation capacity in this study.

4. PCER corrected for temperature in leaves

PCER in leaves located in the upper layer of the canopy was measured in relation to temperature using LI-6400 in December, 2001. Leaf temperature was changed from 15 to 35°C in eight arbitrary steps.

During the measurements, VpdL was 1.7±0.3 kPa, input CO₂ was 370 μmol mol⁻¹, and PAR was 1000 μmol m⁻² s⁻¹, respectively. This data collection was also finished by 11 o'clock.

5. Canopy structure

A 1.0×1.5 m² quadrat in the canopy was divided into five layers 40 cm in thickness, and classified into the upper, middle, and lower layers at 548-508 cm, 508-428 cm, and 428-348 cm in height from the ground, respectively. Leaf areas in each layer were calculated by multiplying the average leaf numbers counted ten times by an average single leaf area measured using an automatic area meter in the laboratory (AAM-7, Hayashi Denkoh Co., Ltd, Japan). Light extinction through the canopy was calculated as the difference (%) between the light intensity at the top and bottom of the canopy. The light intensity was measured using a quantum sensor (LI-190SB, Li-Cor, USA), expressed as an average value measured in each 40 cm layer at five points, the centre and four peripheral points 50 cm away from the centre.

6. Estimation of the size of non-assimilation organ

The above-ground biomass was estimated from the surface area and volume of each organ. *R. stylosa* stands in the study site were designated as protected species, and thus it was impossible to cut down the whole stand to measure the dry weight. The surface area and the volume of branch and trunk were calculated from the length and diameters at the top and the bottom of each organ, based on an assumption that the organ shape was a cone. Surface area estimation of the trunk was chosen in accordance with the fact that the CO₂ emission from the large-diameter plant tissue is proportional to its surface area, because physiological activity of large-diameter tissue is limited only to the

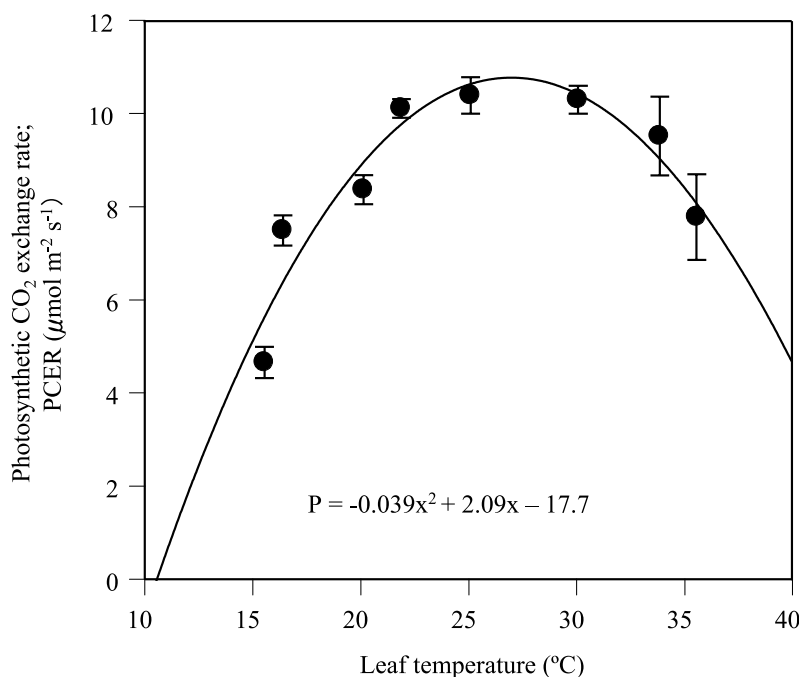


Fig. 2. Influence of temperature on photosynthetic CO₂ exchange rate (PCER) measured in the leaves of *Rhizophora stylosa* in Ishigaki, Japan. A quadratic curve in the graph was a regression curve. Vertical bars are standard deviations.

epidermis and cambium layers (Yoda, 1971).

7. Measurement of RCER in above-ground organs in relation to temperature

Under the government authorization, each representative shoot of the stands located in the upper, middle, and lower layers of the canopy was cut down to measure RCER of branch and leaf. The branch was divided into four offshoot groups; i.e. the primary offshoot attached to the trunk, the branching from the first offshoot, the branching from the second offshoot, and other twigs were named as fourth offshoot. Divided branches and leaves in each layer were kept in refrigerator until the measurement of RCER.

Dark respiration rate of the leaf at the annual average temperature was measured using LI-6400. During the measurement, leaf temperature was maintained at 25°C, VpdL was 1.5 ± 0.1 kPa, CO₂ concentration in the reference air was $370 \mu\text{mol mol}^{-1}$, and PAR was $0 \mu\text{mol m}^{-2} \text{s}^{-1}$. RCER of the trunk was also measured using LI-6400 with an arranged conifer chamber (LI-6400-05, Li-Cor, USA) attached to the trunk surface. Measurement conditions were the same as those for the leaves except that air temperature inside the chamber was 28.0°C. Edges of the chamber were sealed with soft clay. Measurements were replicated at 5 points around the breast height of the trunk.

The RCER of each above-ground organ was measured using a CO₂ gas analyzer (LI-800, Li-Cor, USA). Sample

temperature was controlled from 20 to 35°C at 5°C intervals. The samples were put into aluminous pan (18 cm in diameter and 18 cm in depth), which was kept inside a water bath with immersion thermo-regulator (NTT-1200, Tokyo Rikakikai Co., Japan). Air inside the pan was mixed with a propeller fan (MD825BM-12, Tokuden Co., Ltd., Japan) to maintain a constant temperature. The temperature was measured with a cooper-constantan thermocouple ($\phi=0.32$ mm) attached to the sample surface and recorded with an analogue recorder (EPR-3521, TOA DKK, Japan). The measurement was held for at least 5 minutes to obtain a stable data of linearly increasing CO₂ concentrations.

8. Estimation of absorption and emission of CO₂ without temperature modification

The diurnal change in photosynthetic CO₂ absorption without temperature modification was calculated as an integration of PCER measured at 25°C, and leaf distribution and the light extinction in the canopy. The diurnal fluctuation of light intensity each month was assumed to follow a sine curve, and a shape of the sine curve was determined using the monthly average of the maximum light intensity and the day length (Monteith, 1965):

$$R_t = R_{\max} \cdot \sin\left(\frac{t}{L} \cdot \pi\right) \quad (1)$$

where, R_t ($\mu\text{mol m}^{-2} \text{s}^{-1}$) is the light intensity at a given time, t (hr) after sunrise, R_{\max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) is the monthly average of the maximum light intensity

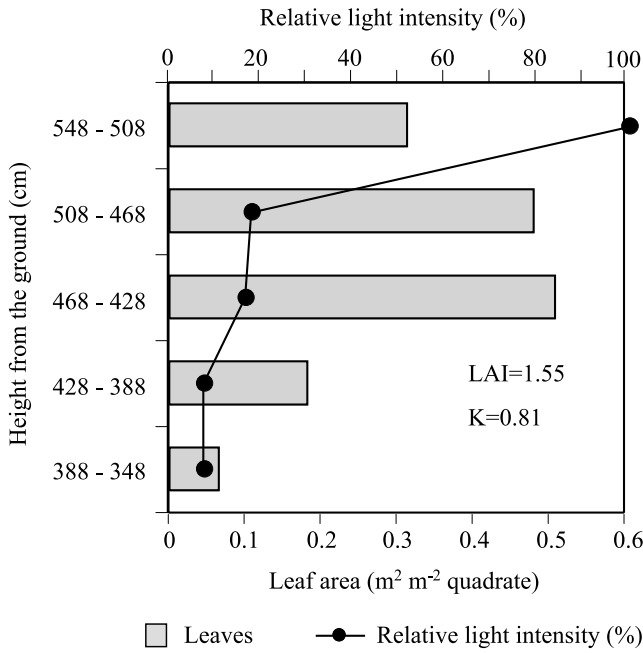


Fig. 3. Leaf distribution and light profile in the canopy of *Rhizophora stylosa* in Ishigaki, Japan. LAI and K in the graph show leaf area index and a light extinction coefficient, respectively.

and L (hr) is the average day length of each month. The light intensity in each canopy layer was calculated by multiplying relative light intensity in each layer by the calculated values of R_e . PCER in the leaf was calculated by assigning the light intensity at each layer (Fig. 1) to part I of Eq. 4.

Respiratory CO₂ emission from whole leaves of the canopy without temperature modification was calculated as dark respiration in a single leaf measured at 25°C, total leaf area of the stand, and monthly dark period from sunset to sunrise. The CO₂ emission without temperature modification from the branch and trunk was calculated by multiplying the average value of RCER at around 25°C by each biomass for a single stand. The RCER per unit surface area was used to estimate CO₂ emission from the branch and trunk.

9. Model for diurnal change of temperature

Diurnal temperature fluctuations each month were calculated with the following equations, which were developed in this study for the day and night. The fluctuation during the day was modeled following the odd function:

$$T_d = f(T_{\max}, T_{\min}, t, t_m, A)$$

$$= \frac{T_{\max} - T_{\min}}{(t_m - A)^2 \cdot \sin\left(\frac{t_m - A}{A}\right)} \cdot (t - A)^2 \cdot \sin\left(\frac{t - A}{A} \cdot \pi\right) + T_{\min} \quad (2)$$

where, T_d is the temperature (°C) at a given time, t (hr) after sunrise (t_r), T_{\max} is the average maximum

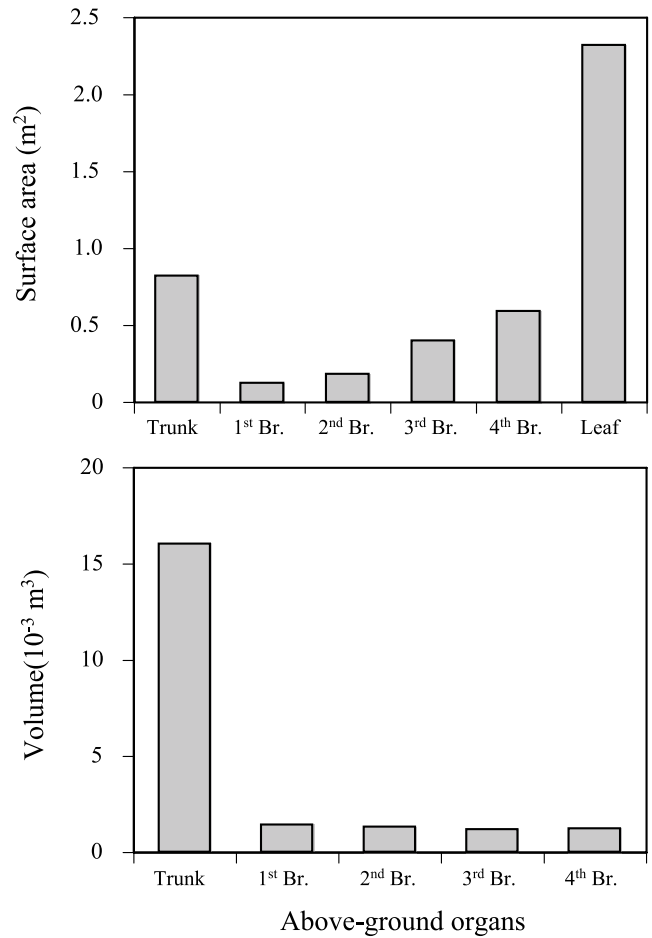


Fig. 4. Surface area and volume of non-assimilation organs in a single stand of *Rhizophora stylosa* in Ishigaki, Japan. "Br." in the graph means branch divided into four offshoot groups; first, second, third, and fourth offshoots.

temperature (°C) of each month at midday (t_m), T_{\min} is the average minimum temperature (°C) of each month, and A is the modified day length (hrs) which was the sum of the average monthly day length (L) and an arbitrary time (γ).

The temperature fluctuation during the night was modeled following the simple exponential function:

$$T_n = f(t_n, B, C) = B \cdot t_n^{-C} \quad (3)$$

where, T_n is the temperature (°C) at a given time, t_n (hr) after sunset (t_s), and B and C are coefficients. The coefficients of Eq. 3 were calculated with the simultaneous equation using the temperature data of T_{\min} at sunrise (t_r) and sunset (t_s). According to the meteorological data provided by Japan Meteorological Agency (<http://www.data.kishou.go.jp/index.htm>), monthly average of the diurnal changes in temperatures is shown.

Results and Discussion

1. PCER in relation to light intensity

Fig. 1 shows the change of PCER in relation to light intensity measured at a leaf temperature of 25°C. The

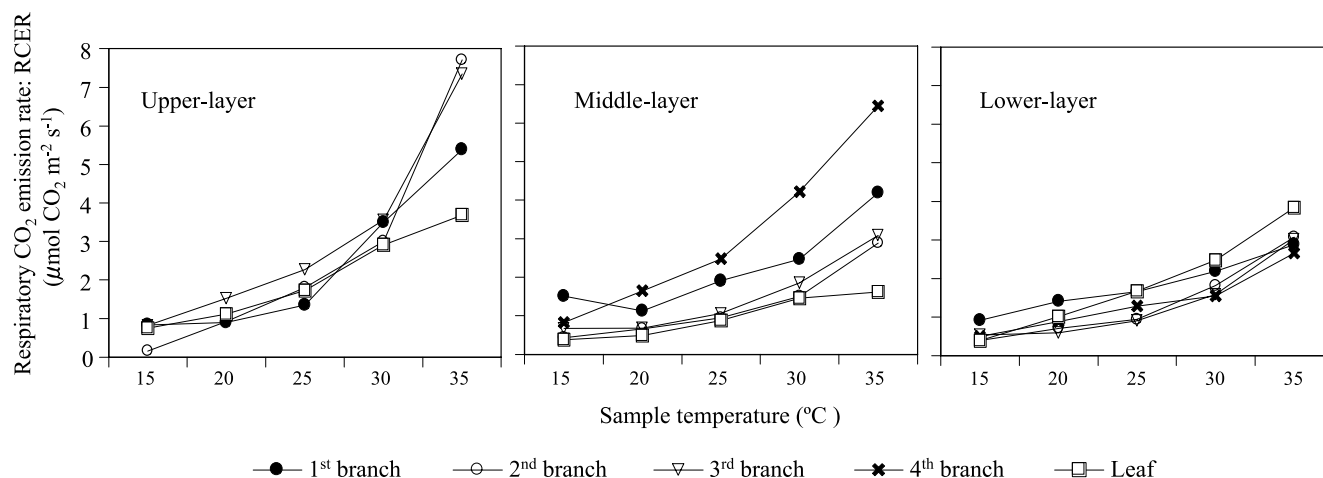


Fig. 5. Influence of temperature on respiratory CO_2 emission rate (RCER) of each organ in three layers of *Rhizophora stylosa* canopy in Ishigaki, Japan. The values of RCER in the graph were calculated per unit surface area of each organ. The branch was divided into four offshoot groups; first, second, third, and fourth offshoots.

response of PCER to light was different with the layer of the canopy. Maximum photosynthetic rates (P_{\max}) increased from the top to the bottom of the canopy as follows; 2.40, 4.47, and $8.87 \mu\text{mol m}^{-2} \text{s}^{-1}$ in upper, middle, and lower layers of the canopy, respectively. The photosynthetic capacity of the leaf in lower layer was 3 to 5 times higher than that in upper layer (Fig. 1). P_{\max} in this study was slightly lower than that in *R. stylosa* in Australia ($12.9 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Clough, 1998). The decrease of PCER from the top to the bottom of the canopy was contrary to the results obtained from *R. apiculata* in Malaysia reported by Clough et al. (1997) (Fig. 1). They reported that the average values of net photosynthetic rates in the leaves of *R. apiculata* decreased linearly from $10.9 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the top of the canopy to $4.9 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the bottom. Our results may be caused by some environmental stress. It was noted that photosynthetic rates of mangroves decreased under less favorable conditions, usually associated with high salinity (Sobrado, 1999), low soil-water content (Smith et al., 1989; Lin and Sternberg, 1992), or exposure to continuous high solar radiation (Clough and Sim, 1989).

The PCER in response to light intensity in the three layers corresponded well to the hyperbolic equation:

$$P = \frac{I}{\alpha + \beta \cdot I} \quad (4)$$

where, P is the PCER ($\mu\text{mol m}^{-2} \text{s}^{-1}$) of individual leaves at light intensity I ($\mu\text{mol m}^{-2} \text{s}^{-1}$), and α and β are coefficients.

2. PCER in relation to temperature

Fig. 2 shows the PCER in relation to temperature measured at PAR of $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$. P_{\max} of $10.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ was obtained at a leaf temperature of 25.1°C . The P_{\max} obtained in the effect of temperature

was 4.3 times higher than that obtained in the effect of light (Fig. 1), although they were measured on similar leaves located in the upper layer of the canopy. The difference in P_{\max} may be due partly to seasonal variation. The effect of temperature was measured in December, while the effect of light was measured in September. This seasonal shift in gas exchange characteristics was similar to that observed in *Rhizophora* species (Moore et al., 1973).

The PCER was in the range of 10.1 to $10.3 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 21.4 to 30.1°C . These temperatures would be an optimum range for the PCER in *R. stylosa* leaves. These temperature ranges were consistent with those of *Rhizophora mangle* reported by Moore et al. (1973).

The PCER decreased from the P_{\max} ($10.4 \mu\text{mol m}^{-2} \text{s}^{-1}$) to 8.4 and $9.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ at a leaf temperature of 20.1 and 33.8°C , respectively. The value of PCER at 30°C ($10.3 \mu\text{mol m}^{-2} \text{s}^{-1}$) was two times higher than that below 15°C ($4.7 \mu\text{mol m}^{-2} \text{s}^{-1}$), suggesting that the photosynthetic performance of leaves varies largely with the ambient temperature. The temperature response curve is represented with a least square fitting as the quadratic curve (Fig. 2):

$$P = -0.04x^2 + 2.09x - 17.7 \quad (5)$$

where, P is the PCER at leaf temperature of x ($^\circ\text{C}$). The response to temperature in leaves in the middle and lower layers of the canopy was assumed to be similar to that in leaves in the upper layer of the canopy.

3. Canopy structure

Fig. 3 shows the relationship between leaf area and light extinction in the canopy. The leaf area index (LAI) was 1.55, which was much lower than that of *R. apiculata* in Malaysia (4.9 to 5.4) (Clough et al., 1997) and *Rhizophora* trees in Australia (3.1) (Clough, 1998). About 80% of light was absorbed or reflected in the top layer of the canopy. Light extinction coefficient

Table 1. Parameters of regression equation ($Y=H \cdot 10^{J \cdot X}$) that show the relationship between temperature (X) and respiratory CO₂ emission rate (RCER, Y). *H* and *J* are coefficients. Values for the branch and leaf were calculated using the RCER per unit surface area ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), whereas those for the sprout were calculated using the RCER per unit dry weight ($\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$). *R*² is a coefficient of determination. *, significant at 5% level.

	Branch				Sprout	Leaf
	1st	2nd	3rd	4th		
Upper layer						
H	0.14	0.02	0.17	—	0.96	0.22
J	0.04	0.08	0.05	—	0.37	0.04
R ²	0.92	0.95	0.99*	—	0.96*	0.99*
Middle layer						
H	0.49	0.10	0.16	0.20	0.96	0.11
J	0.03	0.04	0.04	0.04	0.32	0.04
R ²	0.80	0.99*	0.93	0.99*	0.97*	0.96*
Lower layer						
H	0.43	0.09	0.12	0.17	0.67	0.09
J	0.02	0.04	0.04	0.03	0.35	0.05
R ²	0.98*	0.99*	0.95	0.98**	0.97*	0.96*
Averages in three layers						
H	0.32	0.05	0.15	0.12	0.43	0.14
J	0.03	0.06	0.04	0.04	0.07	0.04
R ²	0.95	0.99*	0.98*	0.99*	0.98*	0.99*

(K) was obtained from the cumulative leaf area and logarithms of relative light intensities in each layer. The value of K in this study (0.81) was larger than that obtained in the canopy of *R. apiculata* in Malaysia (0.54) (Clough et al., 1997) and that in the *R. apiculata* canopy in Australia (0.53) (Clough, 1998). This result suggests that light penetration into the *R. stylosa* canopy was not sufficient as compared to the other mangrove forests.

4. Above-ground biomass

The surface area and volume of each organ in a single stand were calculated and used to estimate the above-ground biomass of the stand (Fig. 4). The leaf area, 2.32 m², was the largest among the surface area of all organs. Total surface area of the branch surface was 1.29 m². This was 57% larger than that of the trunk. Volume of the trunk was $16.0 \times 10^{-3} \text{ m}^3$, and was the largest among all organs. Total volume of the branch was $5.08 \times 10^{-3} \text{ m}^3$, this was about 30% of the trunk volume. Dry weight biomass in one stand was 7.73 kg DW/stand, this was estimated by integrating dry weight of each organ in one stand. The dry weight of each organ was calculated from the surface area and dry weight obtained with the samples used for respiration measurement.

5. RCER and its response to temperature in each organ

The average branch RCER value at 25°C was $1.13 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. The trunk RCER at 28.0°C was from 0.19 to $1.92 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. The average leaf RCER value at 25°C measured using LI-6400 was $0.43 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$.

Fig. 5 shows the effect of temperature on the RCER per unit surface area in branch and leaf. A raise in temperature increased the RCER in all samples. In the range of 15 to 35°C, the branch RCER was 0.84 to $7.71 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, and that of leaf was 0.38 to $3.84 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. The leaf RCER value was similar to that of $1.5 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ reported by Ong et al. (1995).

Rate of increase of RCER by the increase in temperature was higher in the samples of upper layer compared with those of middle and lower layers of the canopy (Fig. 5). At the lower temperatures of 15 to 20°C, there was little difference in RCER among the samples. At the higher temperatures from 30 to 35°C, on the other hand, the RCER of the branches in the upper layer were two times higher than that in middle and lower layer. This result suggests that the branches in upper layer had a high sensitivity to the temperature increase.

The influence of temperature on RCER was well regressed with an exponential equation:

$$Y = H \cdot \exp^{J \cdot X} \quad (6)$$

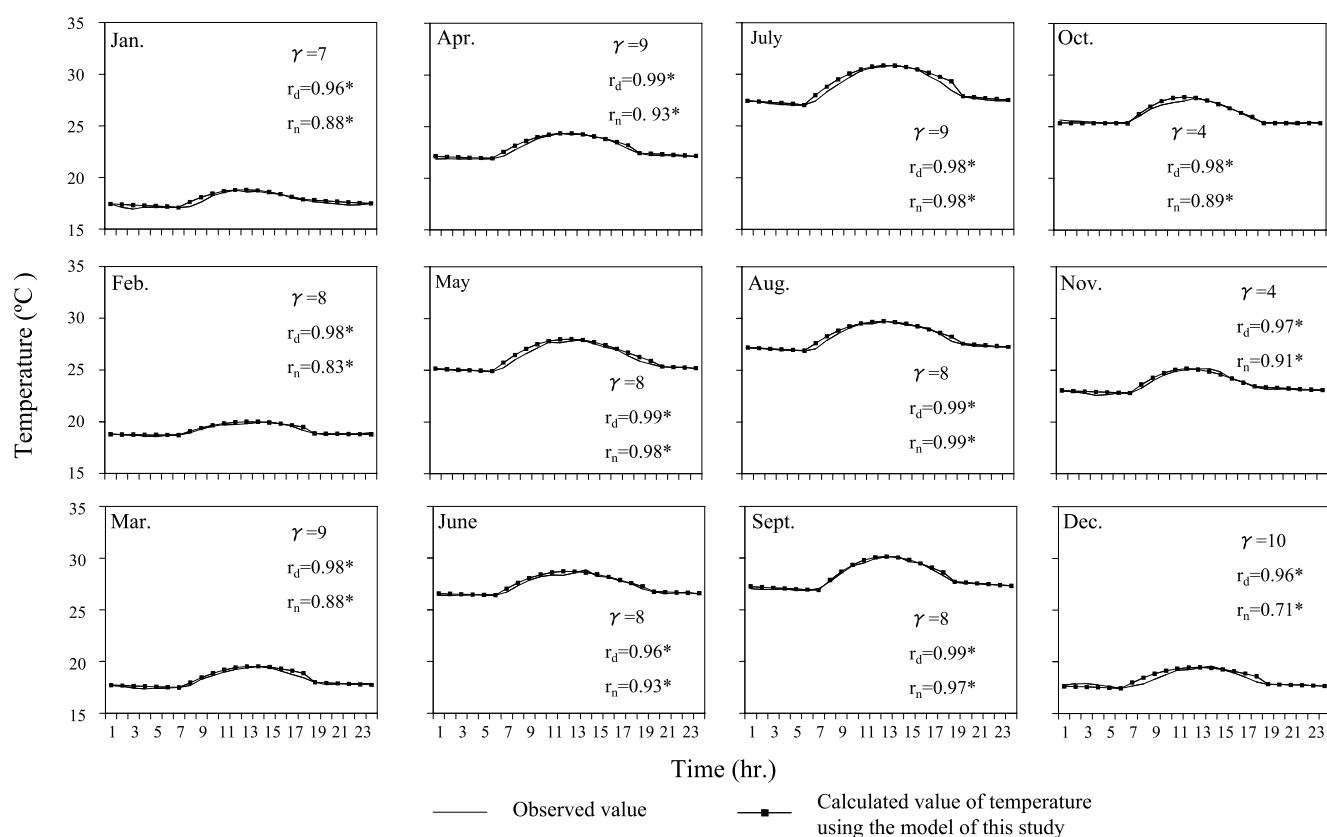


Fig. 6. Comparison of the diurnal variation of temperature in each month between the observed data and that calculated by the model of this study. Temperature variations during the day and night were calculated with Eq. 2 and Eq. 3, respectively. “ γ ” in the graph is arbitrary time to determine the modified (A) from the day length (L) in Eq. 2. “ r_d ” and “ r_n ” are correlation coefficients of the temperature between observed data and calculated value during the day and night, respectively. *, significant at 1% level.

where, Y is the RCER ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), X is the sample temperature ($^{\circ}\text{C}$), and H and J are coefficients. We obtained the regression equation for each separated branch, sprout, and leaf (Table 1). In order to estimate the CO_2 emission from the branch, one regression formula was derived by averaging PCER values obtained from each separated branch of three layers in the canopy (Table 1). In this study, it was not possible to measure the influence of temperature on RCER in the trunk. The CO_2 emission from the trunk was thus calculated by substituting the regression curve obtained for the 1st branch.

6. Model for diurnal temperature change

Modified sine curve such as cubic sine function has been used previously to model the diurnal change of temperature (Seino et al., 1981), but the calculated values did not represent the real diurnal change of temperatures. In this study, we developed the model for diurnal change of temperature as Eq. 2 and 3. The diurnal change of temperature derived from the model was well approximated with that of the real values in each month (Fig. 6). The temperature model includes the value A which shows the modified day length (hr), the sum of the day length (L), and an arbitrary time (γ)

(Eq. 2). The arbitrary value γ for each month appeared to change by 4 to 10 hours (Fig. 6). Modification of CO_2 fixation for a temperature change was conducted using the temperature fluctuation model obtained for each month.

7. Estimation of photosynthetic CO_2 absorption

Photosynthetic CO_2 absorption in the whole canopy was calculated by integrating PCER in each layer. Fig. 7a shows the seasonal variation of CO_2 absorption without temperature modification, which was calculated from the PCER obtained at 25°C . Monthly values of CO_2 absorption increased in the summer season and decreased in winter. This variation was caused by the change in the day-length of each month. A slight decrease in the CO_2 absorption was noted in February because it is a shorter month. Annual CO_2 absorption in *R. stylosa* forests was estimated as $30.0 \text{ tCO}_2 \text{ ha}^{-1} \text{ yr}^{-1}$ (Table 2). This value was comparable to that in mixed mangrove species in Papua New Guinea (16 to $26 \text{ kg C ha}^{-1} \text{ day}^{-1}$, i.e. 21.4 to $34.8 \text{ tCO}_2 \text{ ha}^{-1} \text{ yr}^{-1}$), which was estimated by the traditional gas exchange method (Bunt et al., 1979). This result showed that the gas exchange analysis in this study corresponded with a previous estimation of mangrove productivity.

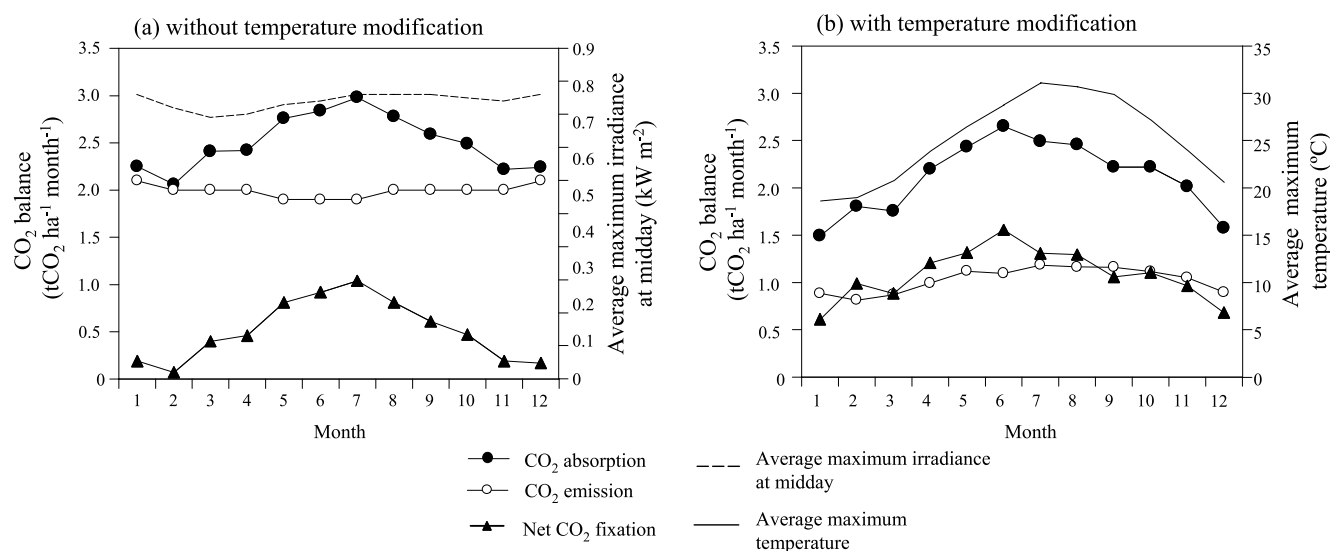


Fig. 7. Seasonal variations of CO₂ absorption and emission in *Rhizophora stylosa* trees in Ishigaki, Japan. Graph (a) shows the CO₂ values calculated without temperature modification (corrected only for the light intensity). Graph (b) shows the CO₂ values calculated with temperature modification (corrected for both the light intensity and temperature).

To the CO₂ absorption rate corrected only for the light intensity, we added temperature modification to increase the accuracy of the estimation of CO₂ absorption rate. The PCER at a given time of the day was corrected for both the light intensity and temperature calculated by Eq. 1, 2, 4, and 5. Fig. 7b shows the seasonal variation of CO₂ absorption rate calculated with the temperature modification. With temperature modification, the maximum value of monthly CO₂ absorption was 2.65 tCO₂ ha⁻¹ observed in June, while the minimum value was 1.49 tCO₂ ha⁻¹ in January. In contrast, the results without temperature modification were 2.98 and 2.06 tCO₂ ha⁻¹ in July and February, respectively (Fig. 7a). The monthly values of CO₂ absorption with temperature modification were 7 to 34% lower than those without temperature modification throughout the year. The pattern of seasonal variation was similar in both cases, but the variation was larger in the values with temperature modification than those without temperature modification. The annual value of the CO₂ absorption was 25.3 tCO₂ ha⁻¹ yr⁻¹ (Table 2). The annual CO₂ absorption with the temperature modification was 15% lower than that without temperature modification. This may be due to an overestimation of CO₂ absorption by omitting temperature modification, which was calculated from PCER values at the optimum temperature of 25°C.

8. Estimation of respiratory CO₂ emission

Respiratory CO₂ emission in above-ground organs was estimated by integrating CO₂ emission from each organ such as trunk, branch and leaf, and multiplying it by total biomass of each organ. Fig. 7a shows the seasonal variation of the monthly CO₂ emission,

calculated from the RCER obtained at the annual average temperature of 25°C. The monthly values of CO₂ emission were changed slightly in the range from 1.92 to 2.07 tCO₂ ha⁻¹ month⁻¹, which might be caused by the monthly change in the night duration. Annual values of CO₂ emission were estimated as 23.9 tCO₂ ha⁻¹ yr⁻¹ (Table 2).

Fig. 7b shows the seasonal variations of the CO₂ emission calculated with the temperature modification. The maximum and minimum value of monthly CO₂ emission calculated with temperature modification was 1.19 tCO₂ ha⁻¹ in July and 0.81 tCO₂ ha⁻¹ in February. By contrast, the values calculated without temperature modification were 2.07 in December and 1.92 in June (Fig. 7a). The monthly value of CO₂ emission decreased by temperature modification by from 38 to 59%. The seasonal variation of the CO₂ emission with temperature modification was more pronounced than that without temperature modification. The annual value of the CO₂ emission was estimated as 12.4 tCO₂ ha⁻¹ yr⁻¹ (Table 2), which was 48% lower than that obtained without the temperature modification. These results suggest that the CO₂ emission calculated without temperature modification may be twice as high as that calculated with temperature modification.

Clough et al. (1997) reported that 22% of the total annual net daytime photosynthetic production is respired by the foliage in the night. The ratio of the foliage respiration in this study was 23.8% without temperature modification, which was similar to the result by Clough et al. (1997). However, the value was increased to 42.7% by temperature modification.

9. Estimation of net CO₂ fixation capacity

Fig. 7 shows the seasonal variations of the CO₂

Table 2. Estimations of annual CO₂ fixation estimated without and with temperature modification and dry matter accumulation in above-ground of *Rhizophora stylosa* forest in Ishigaki, Japan. The dry matter accumulation was calculated by multiplying the value of net CO₂ fixation by the molar mass of carbohydrates (CH₂O=30).

CO ₂ balance	Absorption	Emission	Net fixation	Dry matter accumulation
	tCO ₂ ha ⁻¹ yr ⁻¹	tCO ₂ ha ⁻¹ yr ⁻¹	tCO ₂ ha ⁻¹ yr ⁻¹ (tC ha ⁻¹ yr ⁻¹)	ton ha ⁻¹ yr ⁻¹
Calculated values without temperature modification	30.0	23.9	6.10 (1.66)	4.19
Calculated values with temperature modification	25.3	12.4	13.0 (3.55)	8.90

fixation calculated without and with the temperature modification. Annual values of the net CO₂ fixation without and with temperature modification were 6.10 and 13.0 tCO₂ ha⁻¹ yr⁻¹ (1.66 and 3.55 tC ha⁻¹ yr⁻¹), respectively (Table 2). Estimated values of the net CO₂ fixation were doubled by including the temperature modification. The net CO₂ fixation and carbon accumulation values calculated with temperature modification, however, were 29 to 94% lower than those estimated by the allometric method in *R. apiculata* forests in Thailand and Malaysia (Christensen, 1978; Ong et al., 1984, 1995; Ong, 1993). The CO₂ absorption was also 76% lower than that in mixed mangrove forests, *R. stylosa*, *R. apiculata*, and *Ceriops australis* in Australia (29 tC ha⁻¹ yr⁻¹, i.e. 106 tCO₂ ha⁻¹ yr⁻¹) (Clough, 1998) and 88% lower than that in *R. apiculata* in Malaysia (56 tC ha⁻¹ yr⁻¹, i.e. 205 tCO₂ ha⁻¹ yr⁻¹) (Clough et al., 1997) (Table 2). These are due partly to the lower value of LAI in the study site as described above. The small amount of net CO₂ fixation and CO₂ absorption in this study agrees with the general observation that decreasing growth rate of mangroves with increasing latitude along the eastern coastline of Australia, in association with a decrease in average and minimum temperatures of both air and soil (Clough, 1992).

10. Temperature modification in gas exchange analysis

An estimation of the net CO₂ fixation capacity was doubled by including temperature modification in its calculation, compared with that calculated without temperature modification. Although mangroves grow over a wide range of temperatures, it is surprising that so little quantitative information is available on the effect of temperature on net primary production and growth (Clough, 1992). If the temperature modification could be applied for the estimation of mangrove productivity by the gas exchange analysis, previously published results of the mangrove productivity estimated by the gas exchange method may be upwardly revised.

Accuracy of estimating the CO₂ fixation could be further improved by the following two procedures.

The first is to include temperature profiles inside the canopy in the temperature modification. Ong et al. (1995) reported that the temperature at the top of the canopy was about 10°C higher than that at ground level. This suggests that the temperature value used in this study is substantially higher than the actual temperature in the canopy, causing an overestimation of CO₂ emission. In this respect, it is also necessary to improve the accuracy of the estimation of photosynthetic CO₂ absorption. The second is to treat the samples of each organ for the measurement of RCER with NaClO (Nobel and Palta, 1989; McKee, 1996; Bryla et al., 2001). This procedure was done to omit excess CO₂ emission from bacteria attached on the surface of the tissues (Alongi, 1989; Clough, 1992). These procedures will improve the accuracy to estimate the net CO₂ fixation capacity. We did not estimate root respiration in this study, but we are now trying to estimate it with excavated root samples using the LI-800 (Li-Cor, USA).

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