Fw: Submission Confirmation for Study on Photosynthetic Responses and Chlorophyll Fluorescence in Rhizophora mucronata seedlings under Shade Regimes

Dari: 野瀬　昭博 (nosea@cc.saga-u.ac.jp)

Kepada: 11974006@edu.cc.saga-u.ac.jp; zia_uul@yahoo.com

Tanggal: Jumat, 28 Juni 2013 pukul 13.30 WIB

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野瀬昭博 佐賀大学 農学部 応用生物科学科 〒840-8502 佐賀市本庄町 1 番地
Akihiro NOSE, Fac. Agri. Saga Univ. 1 Honjo-machi, Saga, 840-8502 JAPAN
TEL(+81)952-28-8724(Direct) FAX(+81)952-28-8737(Lab)
****

----- Original Message -----
From: "Editorial Office Acta Physiologiae Plantarum"
<grzegorz_marszalkowski@sggw.pl>
To: "Akihiro Nose" <nosea@cc.saga-u.ac.jp>
Sent: Friday, June 28, 2013 1:18 PM
Subject: ACPP: Submission Confirmation for Study on Photosynthetic Responses and Chlorophyll Fluorescence in Rhizophora mucronata seedlings under Shade Regimes

> Dear Dr Nose,
>
> Your submission entitled "Study on Photosynthetic Responses and
> Chlorophyll Fluorescence in Rhizophora mucronata seedlings under Shade
> Regimes" has been received by journal Acta Physiologiae Plantarum
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> You will be able to check on the progress of your paper by logging on to
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> assigned.
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> Thank you for submitting your work to this journal.
>
> Kind regards,
>
> Editorial Office
> Acta Physiologiae Plantarum

Fw: Your manuscript entitled Study on Photosynthetic Responses and Chlorophyll Fluorescence in Rhizophora mucronata seedlings under Shade Regimes

Dari: 野瀬　昭博 (nosea@cc.saga-u.ac.jp)

Kepada: zia_uul@yahoo.com

Tanggal: Selasa, 5 November 2013 pukul 05.57 WIB

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野瀬昭博　佐賀大学農学部
TEL0952-28-873 Fax0952-29-8737
＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊
----- Original Message -----
From: "Zoltan Gombos" <gombos@brc.hu>
To: "Akihiro Nose" <nosea@cc.saga-u.ac.jp>
Sent: Monday, November 04, 2013 10:01 PM
Subject: ACPP: Your manuscript entitled Study on Photosynthetic Responses and Chlorophyll Fluorescence in Rhizophora mucronata seedlings under Shade Regimes

> Ref.: Ms. No. ACPP-D-13-00947
> Study on Photosynthetic Responses and Chlorophyll Fluorescence in
> Rhizophora mucronata seedlings under Shade Regimes
> Acta Physiologiae Plantarum
>
> Dear Dr Nose,
>
> Reviewers have now commented on your paper. You will see that they are
> advising that you revise your manuscript. If you are prepared to undertake
> the work required, I would be pleased to reconsider my decision.
>
> The reviewers' comments can be found at the end of this email or can be
> accessed by following the provided link.
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> Your revision is due by 02-02-2014.
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> Author. You will see a menu item call Submission Needing Revision. You
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>
> Please note that this letter is a recommendation only, and the final
> decision is the sole responsibility of the Editor-in-Chief.
>
> Yours sincerely
>
> Grzegorz Marszalkowski
> Editorial Office
> Acta Physiologiae Plantarum
>
> Reviewers' comments:
>
> Reviewer #1: Drastic revision
>
> Major concerns: How were the values of the different measured parameters
> obtained by ending up 1 value/month? Are they the average of daily
> measurements?
>
> Te paper gives the HL, ML, and LL values for a July day. But I guess the
> light intensity also changed over the year, and even from day to day. Was
> this taken into account?
> How was the average monthly temperature calculated?
> Also the proportion of the light/dark periods of the days varies
> considerably over the year, was that taken into account?

>
> These conditions can all affect the values of the observe parameters, and
> might be responsible for some of the small changes, which are interpreted
> in the manuscript as real difference.
>
> If there are daily measurements, it might be considered just to plot all
> of them, without averaging the m into monthly data, since several major
> change in the light, temperature is not necessarily coinciding with the
> monthly calendar.
>
> The text of the paper needs very drastic, and careful revision. On many
> places, I made suggestions, corrections, these place are normally yellow
> colored in the text, my suggestions are given in bold face. In addition,
> please check the changes by the
> Tools/Track Canges/Highlight changes of the file. These corrections are
> only suggestions, sometimes only guesses about the intention of the
> authors.
> For all the detailed remarks, see the attached file Content b.doc
>
>
>
> Reviewer #2: The authors followed the changes for 1 year in certain gas
> exchange and chlorophyll-a fluorescence induction parameters in Rhizophora
> mucronata seedlings under different light conditions. Although the work is
> mainly descriptive, it can be accepted after the following modifications:
> - The authors should clearly show the importance of this work together
> with the new findings in a "Conclusions" chapter.
> - Besides the photosynthetic processes, acclimation to various light
> intensities may have an influence on several physiological processes,
> including acclimation mechanisms, which are not directly related to
> photosynthesis. To get a more global picture based on the present results,
> this fact should also be discussed (See for example: Gray et al., 1997,
> Plant Physiology: they showed first a light-dependent induction of a gene
> earlier related to cold induction; or Majláth et al. 2012 Physiologia
> Plantarum: they provided a complete microarray-based gene expression
> analysis of light-dependence of genes together with hormonal analyses.)
> - the description of statistical analysis is missing.
>
> __
> There is additional documentation related to this decision letter. To
> access the file(s), please click the link below. You may also login to the
> system and click the 'View Attachments' link in the Action column.
> http://acpp.edmgr.com/l.asp?i=81665&l=13WCQ5KR

Introduction

Mangroves represent an important coastal ecosystem in the tropic area because of their high productivity and adaptation ability under various abiotic stresses. Subjects of daily, monthly, and annual variations in their physical environment, mangroves have a remarkable ability to cope with stress conditions (McLeod and Salm 2006). Light, salinity, and flooding are considered as the dynamic stressors in mangrove habitat.

Adaptation in shade tolerance is one of some causes of mangrove distribution patterns (Macnae 1969)**I do not understand this sentence.** ***Maybe: Adaptation to shade is one of the causes of mangrove distribution patterns.*** Significant differences in survival were found among mangrove species, between intertidal zones and due to light level (Smith 1987). ***Maybe: Significant differences in the survival rates of the mangrove species were found depending on their intertidal positions and light exposition.*** One hypothesis claimed that shade intolerance of mangrove seedlings was an additional stress on the ever-present stressor, salinity (Janzen 1985). In contrast, Smith (1987) stated that the influence of light did not appear to be as influential as hypothesized. Although significant light effects were found **(in what respect, if the growth and survival differences were small?,** the differences in growth and survival of seedlings grown in light or in shade were small. Furthermore, the different of light requirements among mangrove species indicated light-dependent responses of photosynthetic rate (Clough 1998). **I do not understand this sentence. In addition, it should be also said what were the light-dependent responses (increases, decreases of what, etc. The sentence has no information this way)**

Mangroves belong to the group of plants, which use C_3 photosynthesis that might show differences in photosynthetic capacity and sensitivity to environmental conditions for different species (Ball 1986). ***Maybe?:*** As regards light competition, gas exchange and chlorophyll fluorescence characteristics of mangrove *Avicennia marina* is typical of sun leaves (Ball and Crithcley 1982). On the other hand, *Bruguiera sexangula* responded favourably to short burst of sunlight at low light level and ***Maybe?:***is considered as relatively shade tolerant species (Krauss and Allen 2003).

Rhizophora mucronata Lamk, "the intermediate gap-phase mangrove species", is found worldwide from East Africa and India through Asia as well as Indonesia to the western Pacific, in wet tropical regions of Australia and in Mozambique and South Africa (Hoppe-Speer et al. 2011). In Indonesia, *R. mucronata* commonly found between zonation of *Avicennia* and *Bruguiera* (White et al. 1989 ; Whitten et al. 2000) that occupies a gradient from low intertidal swamp margins with high insulation, to shaded sites at high water. *R. mucronata* had a role as main plant in the reforested thinned site in tropical coastal area (Srivastava et al. 1988) and produced more leaf litter than the reforested unthinned and natural sites (Wang'ondu and Virginia 2010). While thinning activity contribute on shading conditions, information of seedlings adaptive capacity to shade regimes in relation to photosynthetic performances is essential to clarify both the mangrove zonation pattern and the growth model of *R. mucronata* in the restoration area.

Light or shade regimes were considered to affect not only photosynthetic rate but also chlorophyll fluorescence. Exposure to excess irradiance can lead to photoinhibition, which is characterized by a light-dependent reduction in the fundamental quantum yield of photosynthesis and a loss of photosystem II (PSII) activity (Osmond 1994). So far, there is no specific information about chlorophyll fluorescence of *R. mucronata* seedlings under shade regimes.

The contrasting low- and high-shading areas will create varying combinations of light and temperature also. Ong et al (1995) reported that the temperature on the top of the mangrove canopy was about 10 ^{0}C higher than at the ground surface. The temperature grade is substantially higher than the actual temperature in the canopy, causing an overestimation of CO_2 emission (Okimoto et al. 2007). **I do not understand this sentence.** If a shaded leaf becomes exposed to full sunlight, does its temperature exceed the optimum for photosynthesis? Conversely, what happens to a sun leaf offer any advantage when it is under low temperature? **I do not understand this sentence. Maybe?** *Conversely, what happens with a leaf originally sunned, has the lowering temperature upon shading any advantage for its functioning?* To answer such questions, we also investigated the photosynthetic responses of sunned and shaded leaves of *R. mucronata* seedling under ambient greenhouse temperature for 1 year, while the temperature is different at each months. **I do not understand this part of the sentence!*The ambient greenhouse temperature varied according to the seasons, not? What was then the different temperature in each month?***

Finally, seasonal information of photosynthetic rate and chlorophyll fluorescence in *R. mucronata* seedlings under shade regimes will contribute to a better improving on photosynthetic capacity as estimation of mangrove growth model. **I do not understand this part of the sentence.**

Materials and Methods

Plant materials and growth conditions: Propagules of *R. mucronata* were collected from Galang Island (0° 45' N, 104° 15' E) in Batam District, Indonesia. Propagules were planted in the greenhouse with heating system at the Laboratory of Tropical Crop Improvement, Faculty of Agriculture, Saga University, Japan (33° 14' N, 130° 17' E) on June 2010. After five months, seedlings with 3-4 pairs of leaves were grown under full sunlight (HL), 50% shading (ML) and 80% shading (LL). Shade treatments were done by neutral density black nylon netting. During the experiment, seedlings were watered to ensure that drought did not confound experimental results.

Light intensities were measured on midday at July 20, 2012, a sunny cloudless day, and showed that the actual photosynthetically active radiation (PAR) was 345, 885 and 1728 μmol photon m^{-2} s^{-1}, for LL, ML and HL treatments, respectively. The monthly variation of air temperature in the greenhouse from August 2011 to July 2012, measured with a portable Thermo Recorder equipped with an external thermosensor (TR-50C, T and D co. Ltd., Nagano, Japan), is displayed in Fig 1. **How the monthly values were obtained? I guess, the temperature was recorded continuously then for each day the maximum, minimum and average temperature was determined, and these daily values were averaged over a month to get the data points plotted in Figure 1. Was it so? Please tell something about it in the text.**

Leaf Gas Exchange: The responses of mangrove seedling for leaf gas exchange to shade treatments were evaluated for 1 year from August 2011 to July 2012, beginning after seedlings had been exposed to their shading treatments for 8 months. Net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were measured with a portable open-flow gas exchange system (LICOR 6400, Li-COR, Lincoln, NE, USA). Measurements were made at fully expanded leaves in the morning (08:00 h, local time) until close to mid-day (11:00 h).

Photosynthetic rate under shade regimes was evaluated in relation to light intensity and temperature. In relation to light intensity, PAR value on leaf surfaces was automatically maintained in decreasing order from 1000 to 0 μmol m^{-2} s^{-1} (1000, 500, 250, 100, 50, 0 μmol m^{-2} s^{-1}). During the

measurements, leaf temperature was controlled at 30 ^{0}C, vapour pressure deficit between the leaf and air (VpdL) was 1.7 $\pm$ 0.3 kPa, and CO_2 input was 370 µmol mol^{-1}. The effect of leaf temperature on photosynthetic rate was measured from 20 to 38 ^{0}C under PAR, VpdL and CO_2 input were 1000 µmol m^{-2} s^{-1}, 1.7 $\pm$ 0.3 kPa, and 370 µmol mol^{-1}, respectively. Quantifying the photosynthetic rate as a function of C_i was done by changing the CO_2 concentration at the leaf surface from 0 to 1000 µmol mol^{-1}, under PAR 1000 µmol m^{-2} s^{-1} and leaf temperature 30 ^{0}C.

Chlorophyll Fluorescence: Leaf chlorophyll fluorescence was measured with a modulated chlorophyll fluorometer (OS5-FL, OPTI-SCIENCES, USA) between 08:00 h and 11:00 h, on the same leaves used for gas exchange analysis. The fluorescence parameters were obtained under both dark-adapted fluorescence and yield of energy conversion as described by Genty et al (1989). In leaves submitted to darkness, readings were taken after 30 minutes dark adaptation using a leaf clip. Minimum fluorescence (Fo) was determined by a weak red light and maximum fluorescence (Fm) was induced by a 0.8 s pulse of 2000 µmol m^{-2} s^{-1} PAR. The steady state fluorescence (Fs) was recorded and a second saturating pulse was applied to determine the maximum light-adapted fluorescence (Fm'). A 685 nm light source equipped with OS5-FL was used for the illumination of leaf as actinic light. The actinic light was removed then the minimum fluorescence level in the light-adapted state (Fo') was determined after 10 s of far red illumination. The following chlorophyll fluorescence parameters were calculated according to Genty et al (1989) and Maxwell and Johnson (2000): quantum yield of Photosystem II, ΦPSII = (Fm'-Fs)/Fm'; maximum quantum efficiency of fluorescence PSII, Fv/Fm = (Fm-Fo)/Fm; photochemical quenching coefficient, qP = (Fm'-Fs) / (Fm'-Fo'); non-photochemical quenching, qN = (Fm-Fm') / (Fm-Fo'); and electron transport rate, ETR = ΦPSII x PAR x 0.5 x 0.84. PAR corresponds to the flux density of incident photosynthetically active radiation, 0.5 was as a factor that accounts for the portioning of energy between PSII and PSI, and 0.84 was assumed from an average of 84% of the incident light were absorbed by the leaf.

SPAD reading as representative of relative chlorophyll content was measured by using SPAD-Chlorophyll meter (SPAD 502, Minolta, Osaka, Japan).

Results

Leaf morphology and SPAD value. Shade treatments affected *R. mucronata* leaf morphology. LL leaves were larger than HL and ML leaves. Leaf colour of LL-plants were dark green, while those of ML- and HL-plants were green and light green, respectively (Fig 2).

SPAD readings being in tight correlation with chlorophyll content (Markwell et al. 1995) showed similar HL<ML<LL pattern for each month (Fig 3). The SPAD value of HL and ML leaves was the lowest in February, for LL leaves in July 2012. Furthermore, decreasing SPAD value of HL leaves also occurred on July 2012. **From Figure 3, the tatements of this paragraph cannot be seen, since the errors are considerable. In my view, one can only conclude that: Only HL and ML leaves showed seasonal SPAD value variation, exhibiting a slight minimum around February.**

Effects of light intensity on P_N, g_s, E, and Ci.

Variation of P_N responses to light intensity at 30 ^{0}C of leaf temperature showed almost similar trends for all three treatments, increased simultaneously with PAR escalation until reaching their saturation point (Fig 4).

The light responses of P_N, g_s and E were disclosed using the rectangular hyperbola model (Okimoto et al. 2008; Table 1):

$$P = \frac{I}{\alpha + \beta \cdot I} \qquad (1)$$

where P is P_N of individual leaves at light intensity of *I* (μmol photons m^{-2} s^{-1}), then α and β are coefficients to determine the convexity of the hyperbola. When used to model of conductance and transpiration responses, P was substituted to represent the g_s and E values in Eq.1. HL and ML had higher P_N, g_s and E than LL leaves while PAR increasing.

Equation 1 was used to determine maximum photosynthetic rate (P_{max}), maximum stomatal conductance (g_{max}), and maximum transpiration rate (E_{max}) at saturation conditional (Table 1). The light saturation points of all treatments were commonly at PAR level around 1000 μmol photons m^{-2} s^{-1}. P_N, g_s and E responses to light during hot and sunny months (June-September) tended to increase rapidly up to PAR 100 μmol m^{-2} s^{-1}, had high values and wide gap value between shading treatments at saturation point. In the other side, during cold months (December-March) they were characterized with rapid increasing up to PAR about 250 μmol m^{-2} s^{-1}, low values and no significance difference at saturation point (Fig 4).

Under light saturation, P_{max} showed a positive correlation with g_{max} and E_{max} (Fig 5). The highest values of g_{max} and E_{max} showed similar trends, there were LL<ML<HL respectively. Lower rates of g_{max} and E_{max} for LL leaves probably restricted P_{max}. We found that although the highest value of g_{max} and E_{max} of ML were lower than HL, but their highest P_{max} value were tendency similar.

Effect of temperature on photosynthesis.

The quadratic curves were fitted to describe the temperature responses of P_N (Fig 6). The results showed that relationship between P_{max} and leaf temperature indicated a broad peak for difference season. During mid-high temperature months between August-November 2011 and May-July 2012, P_{max} was obtained at leaf temperature between 29-34 ^{0}C, and decrease at 23-29 ^{0}C on cold months (December 2011-April 2012). P_{max} for the temperature responses of HL (14.9 μmol m^{-2} s^{-1}) and LL (12.0 μmol m^{-2} s^{-1}) occurred on September 2011 at leaf temperature 32 ^{0}C, while ML (13.8 μmol m^{-2} s^{-1}) ensued on July 2012 at 33 ^{0}C**. I do not really understand this paragraph. What was the idea behind these experiments? Since the ambient temperature was varying along the year, the starting temperature of 24 °C was very different as compared to the mid temperature over the given month, therefore the relative "temperature shock" would be markedly different for the leaves. How were the monthly data got in Figure 6?**

We also found that LL leaves sustained a better photosynthetic performance at leaf temperature 25 ^{0}C than HL and ML leaves. In contrast, at leaf temperature 30 ^{0}C, P_N of HL and ML leaves was higher generally than LL leaves (Fig 7). **In the light of the comment above, the problem is the same here, but evidently the leaves are closer to their "normal" state at 30 °C than at 25 °C since only the 30 °C leaves show seasonal variations.**

Effect of C_i on photosynthesis.

The carboxylation efficiency that related with Rubisco activity can be estimated as the initial slope of the response P_N to C_i (Ku and Edwards 1977; Sage and Reid 1994). The initial slope of P_N (C_i) curve is calculated and derived from Eq. 1 while Ci tend to zero, i.e.

$$P = \frac{I}{\alpha + \beta \cdot I}$$

$$P' = \frac{\alpha + \beta \cdot I - \beta \cdot I}{(\alpha + \beta \cdot I)^2}$$, and while *I* toward zero

$$P' = \frac{\alpha}{\alpha^2}$$

$$P' = \frac{1}{\alpha} \quad (2)$$

where P' is initial slope of P_N (C_i) curve and α is first coefficients to determine the convexity of the hyperbola. The carboxylation efficiency implied increasing in photosynthetic rate achieved per unit increasing in CO_2 at the site of CO_2 fixation. Figure 8 showed that initial slope during the hot months were higher than that of the cold months; actually, it declined from August 2011 to March 2012, and went up again until July 2012. It suggested that carboxylation efficiency was higher on hot months compared with cold months. We also found that initial slope of LL leaves was tendency iously lower than HL and ML leaves. **This is true only from April to July!** This result also indicated that carboxylation efficiency of *R. mucronata* leaves were influenced by both temperature and shade regimes.

Chlorophyll fluorescence.

The seasonal variation of quantum yield of PSII (ΦPSII) and electron transport rate (ETR) measured after 30 minutes exhibited the same seasonal variations as the other photosynthetic parameters. The ΦPSII and ETR decreased from August 2011 to February 2012 then increased from March until July 2012. Their lowest values occurred on February 2012. During cold months period (December 2011-March 2012), LL showed relatively high values of ΦPSII and ETR as compared with HL and ML (Fig 9).

Photochemical quenching (qP) is a ratio of light energy used in the transfer of photochemical electrons to total light energy captured by antenna pigment and non-photochemical quenching (qN) reflects a ratio of light energy consumed by heat to the total light energy (Zhou et al. 2010). The highest qP value of HL and LL occurred at September 2011 while ML ensued at July 2012 (Fig 10 a). Unexpectedly, the qP value for HL also high on February 2012,**These are not differences large enough for such a discrimination. There is a slight seasonal variation, but e.g. I see no difference between Aug. 2011 and July, 2012** whereas the P_N and SPAD value were low (Table 1, Fig 3). Furthermore, on September 2011 and between December 2011-February 2012, qN values of HL leaves were higher as compared with other treatments (Fig 10 b). We also found that the qN values of LL leaves usually lower than other treatments during 1 year observation**. I do not understand this sentence, which other treatments?**

A reduction in the ratio of variable to maximum chlorophyll fluorescence (Fv/Fm) can be used as an indication of photoinhibition (Björkman and Demmig 1987; Robakowski 2005). Significant decreasing of Fv/Fm for HL and ML leaves occurred mainly in February and March 2012 while for LL leaves it happened in July 2012 (Fig 11). **Here again, what can be safely said, it is that HL and ML Fv/Fm values showed seasonal variation, the LL practically did not.**

Discussion

The results showed significantly increased SPAD values ($P < 0.05$) and leaf sizes while in plants exposed to 50 and 80% shading (Fig 2 and 3). These results indicate the strategy of *R. mucronata* seedlings to adapt extreme light intensities: HL seedlings decreased their light absorption by reducing chlorophyll content and leaf area; in contrast, LL seedlings increased their light absorption by rising their leaf area and chlorophyll content. Previous studies have shown that plants grown under shaded conditions were noted to increase their pigment density per unit leaf area (Wittmann et al. 2001, Xu et al. 2009), to optimize their height, leaf area, crown extension and leaf arrangement to get the best use of light (Paquette et al. 2007, Huang et al. 2011). When growing in a high-light environment, avoidance of light absorption, e.g. through low chlorophyll contents, played a crucial role in protecting the photosynthetic apparatus of leaves (Adams et al. 2004). We have also found decolouring symptom with lower SPAD value of HL and ML leaves that must have been caused mainly by low temperature in February 2012. Decolouring may occur as a consequence of the combined effects of high incident PAR and low temperature (Close et al. 1999). Especially for HL and ML leaves of *R. mucronata*, these results were in agreement with Kao et al (2004) findings which showed that leaves of mangrove *Avicennia marina* during low temperature at 15 ^{0}C had a greater reduction in chlorophyll content rather than 30 ^{0}C. In the other side, LL leaves had not decolouring symptom during low temperature, it was almost similar with no significance chlorophyll content of mangrove *Kandelia candel* grown at 15 and 30 **^{0}C I do not understand this sentence!** ***You meant? that the LL leaves did not decoloured at low temperature similarly to the case found for Kandelia candel either at 15 or 30 ℃? I am just asking this, as a guess.*** (Kao et al. 2004). Although LL got significance reduction SPAD value on July 2012 but still higher than HL and ML on the same month (Fig.3). **Maybe: Altohugh LL exhibited a significantly reduced SPAD value in July, but this value was still higher than those of the HL and LL leaves in the same period.** We suggest that this is not a decoloring symptom but more than as LL protection mechanism to adapt

with the incident high radiation on July 2012.**??? Decolouring in itself is the sign of adaptation, not? Or can you decide, from the SPAD values, why there are less chlorophyll present, because the light destroyed it, or because the plant removed it/synthesized less?** The reduction of photosynthetic pigments could be seen as a protection mechanism as it would mitigate the capacity of the leaf to absorb incident radiation and therefore demote the amount of excess excitation energy that has to be dissipated (Burritt and Mackenzie 2003).

Significant increases in total chlorophyll lead raising in CO_2 exchange were due to increased photosynthetic capacity (Evans 1989), as shown in mangrove *A. marina* and *Hibiscus tiliaceus* (Naido et al. 2002). However, this study has been unable to demonstrate that higher total chlorophyll had high P_N in *R. mucronata* seedlings under shade regimes. The contrary result showed that HL and ML had higher P_N than LL leaves while PAR increasing (Fig 4). We found that under light saturating conditional, g_{max} and E_{max} showed similar trends, there were LL<ML<HL respectively (Fig 5, Table 1). It described that the P_{max} of *R. mucronata* seedlings were more influenced by g_{max} and E_{max} rather than chlorophyll content. The circulation of CO_2 is determined by stomatal density, size, and conductance (Xuan et al. 2011), and among of those factors, stomatal conductance is the most prominent (Putra et al. 2012). Cheeseman et al (1997) found that the relationship between net CO_2 assimilation and g_s in mangrove *Rhizophora stylosa* was significant and positive while measured under intermediate temperature and high light. Lower rates of g_{max} for LL leaves probably restricted the maximum photosynthetic rate, that similarly as shown at "the shade tolerant mangrove species", *Bruguiera sexangula* (Krauss and Allen 2003). High stomatal conductance was followed by increased transpiration rate. The positive relationships between P_N, g_s and E were also found at mangroves seedlings of *R. stylosa* grown under light levels (Kitaya et al. 2002). Moreover, ability of ML leaves to achieve high P_{max} in lower g_{max} and E_{max} compared with HL leaves, indicate ML effectiveness and also chance to conserve water in better level. It will be useful while ML seedlings adapt with saline conditional.

We found that the light saturation point of all treatments were commonly at PAR level around 1000 μmol photons m^{-2} s^{-1}. These results were higher than mangrove *B. sexangula* and similar with *A. marina.* The finding of Krauss and Allen (2003) estimated that light saturation point of *B. sexangula* seedlings usually below 500 μmol photons m^{-2} s^{-1} under both **LL** and **HL** conditions. The assimilation rates of *A. marina*, "the sunlit mangrove species" became light saturated at approximately 1000 μmol

photons m^{-2} s^{-1} in leaves from understory**???** shade condition and high light regime (Ball and Critchley 1982). It can therefore be assumed that *R. mucronata* leaves are more a sunny leaf type while compared with ***than those of*** *B. sexangula*. This result also elucidate the zonation pattern of *R. mucronata* that common found between zonation of *Avicennia* and *Bruguiera* **I do not understand this sentence!** (White et al. 1989 ; Whitten et al. 2000).

Our finding showing different characteristics of P_N responses of *R. mucronata* leaves to light intensity (Fig 4) in the hot (June-September), and in the cold (December-March) months emphasized the role of temperature for mangrove seedling growth and photosynthetic performances. Low temperature clearly modified the passage of light response curves on cold months compared with hot months.

Photosynthesis of mangroves has been indicated to be highly sensitive to leaf temperature (Andrews et al. 1984; Ball et al. 1988). In view of the ecological distribution of plants, it was necessary to explain the temperature response curve of photosynthesis (Agata et al. 1985), and also could improve the accuracy of estimation of CO_2 fixation capacity by mangrove (Okimoto et al. 2007). Moore et al (1973) reported that P_{max} of mangrove *Rhizophora* and *Laguncularia* was obtained at leaf temperature near or below 25 ^{0}C. In contrast, the latter partly**????** reported that the relationship between the net photosynthetic rate and leaf temperature indicated a wide peak between 29 and 34 ^{0}C (Okimoto et al. 2007). Our finding showed that relationship between P_{max} and leaf temperature indicated a broad peak, which was depending on the ambient temperature. At high ambient temperatures between August-November 2011 and May-July 2012, P_{max} was obtained between 29-34 ^{0}C leaf temperatures, but at lower (23-29 ^{0}C) leaf temperatures in the other months (Fig 6). We also found that LL leaves sustained a better photosynthetic performance at lower leaf temperature as compared to HL and ML leaves (Fig 7). Some studies have found that the optimum temperature for plant photosynthesis depended strongly on their growth-temperature (Sawada and Miyachi 1974; Kao et al. 2004). The temperature is lower in the deep-shade areas than the sun-exposed ones, thus, LL seedlings exhibited better photosynthetic performance at lower temperatures.

Sharkey (1985) pointed out that the rates of photosynthesis were a function of both the simplicity**???** which stomata allow carbon dioxide to penetrate the leaf and the biochemical capacity to fix CO_2. Change in the shape of the P_N (C_i) curve was not only beneficial to indicate variability in the capacity for photosynthesis, but also elucidate which regions of photosynthetic biochemistry are sensitive

to environmental (Ball 1986). Initial slope of the response of P_N to C_i could be correlated to *in vivo* assessment of biochemical components of leaf photosynthesis, such as ribulose-biphosphate carboxylase (rubisco) activity (Caemmerer and Farquhar 1981). As shown in Fig 8, the initial slope of P_N (C_i) curve suggested that carboxylation efficiency was higher on hot months compared with on cold months. In contrast to Sage and Reid (1994) that reported the initial slope P_N (C_i) is little affected by temperature, we found that seasonal variation of temperature significantly affect initial slope. This result was in agreement with Campbell et al (2005) findings which showed increasing temperature increased the initial slope and the maximum rate of assimilation. Furthermore, the low initial slope of LL leaves also supported the lower P_N of LL leaves compared with HL and ML leaves. This result also suggested that the carboxylation efficiency of *R. mucronata* leaves was influenced by shade regimes. Sage and Reid (1994) reported that the changes in the content of the major photosynthetic constituent (PSII content, ATP synthase, rubisco) occur with the greatest rate of adjustment after long-term acclimation to light regimes.

Φ PSII is the proportion of absorbed energy being used in photochemistry (Maxwell and Johnson 2000) that represents the efficiency of energy conversion of open PSII (Schreiber et al. 1994), and ETR represents the relative quantity of electron passing trough PSII during steady-state photosynthesis (Tezara et al. 2003). The reduction of ΦPSII and ETR for all treatments during cold months (Fig 9) were caused mainly by low temperature. Lowering the temperature generally reduces metabolic rates and can therefore limit the sinks for the absorbed excitation energy, particularly CO_2 fixation (Alam et al. 2005). A reduction in chlorophyll fluorescence in response to low temperature has also been observed in mangrove *K. candel* and *A. marina* (Kao et al. 2004). Furthermore, the combination of low temperature-high light intensity conditional during cold months might accelerate the damage to the photosynthetic apparatus (Alves et al. 2002). However, we also found that during cold months (December 2011-March 2012), LL showed relatively high values of ΦPSII and ETR after dark adaptation compared with HL and ML (Fig 10). This finding suggest that the adaptation of LL leaves in dark conditional that characterized with lower temperature rather than grown under light was helpful to protect PSII centre while exposed on low temperature.

The high qP values for all treatments during hot months are useful to sustain the high photochemical capacity. The similar patterns of the highest qP and P_{max} value for each treatments that occurred on same months (Fig 10a and Table 1) demonstrate the contribution of qP in order to P_{max}

achievement level. The response of qP represented the openness of PSII centres (Kitao et al. 2003) and high qP was beneficial for the separation of electric charge in reaction centre (Dai et al. 2009). Furthermore, the high qP value of HL leaves on February 2012 whereas the P_N was low indicate abnormal conditional because of photodamage. Although the mechanism is not clear, during low temperature in cold months, it was possible that photochemical quenching was not contribute to temperature. Normally, a higher in P_N resulted a higher qP in plants (Kao and Tsai 1999).

Moreover, the high qN value of HL leaves on February-March 2012 (Fig 10 b) represented that the using of light energy probably already exceed photosynthetic capability and also level of heat dissipation. qN reflects the amount of energy dissipated by non-photochemical quenching by plants (Liu et al. 2007). While photosynthesis is incapable of using all of the energy absorbed by light-harvesting complexes (Bajkan et al. 2012), the absorbed light energy not utilized in photochemistry is often dissipated thermally (Martin et al. 2010). Furthermore, heat dissipation level that too high might cause "chlorotic" at leaves. It was similar with phenomena of the lowest SPAD value of HL leaves on February-March 2012 (Fig 3). Severely chlorotic leaves might be the result of high light intensity (Olsen et al. 2002; Huang et al. 2011).

The regular value 0.75 - 0.85 of Fv/Fm ratios have been considered normal for unstressed plants (Hunt 2003), and decline of Fv/Fm under 0.75 could indicate a disturbance in or damage to the photosynthetic apparatus that due to photoinhibition (Litchtenthaler et al. 2005). HL & ML got photoinhibition on February and March 2012 (Fig 11), probably was caused mainly of low temperature. Photosynthesis is inhibited by low temperature, in part as an impact of reversible or reversible damage to photosynthetic structures (Robakowski 2005). The combination of low temperature and high light may affect leaf membranes and destruct the photosynthetic apparatus of higher plants (Krause 1994). Furthermore, chronic photoinhibition of HL and ML leaves might cause decoloring of photosynthetic pigments such as chlorophyll and carotenoids (Powles 1984; Takahashi et al. 2002).

In contrast with some studies that reported photoinhibition tend occurred when shade-adapted plants were exposed to high-light stress (Khan et al. 2000, Xu et al. 2009), we found that LL sustain low susceptibility photoinhibition. In this study, although Fv/Fm of LL leaves decline during cold months and shinning months, but the values were higher than 0.75 (Fig 12) and also never show chronic photoinhibition level. LL seedlings might have the ability to maintain photosynthetic activity in response to low temperature, non-freezing temperature, because of their protection mechanisms. The response of

plants grown in darkness to low temperature had little effect on the PSII complex compared with under light (Alves et al. 2002). Furthermore, we suggested that the decreasing Fv/Fm of LL leaves during shinning months July 2012 simultaneously with reducing of SPAD value (Fig 3) as a mitigation strategy of the leaf to absorb incident radiation and therefore demote the quantity of excess excitation energy that has to be dissipated. Although reducing of SPAD value occurred on July 2012, but the photosynthetic performance of LL seedling was not decline (Fig 4). However, this result was also in agreement with Pompelli et al (2010) and Huang et al (2011) findings which showed that photoinhibition was not found in plants grown in shade area. Currently, we are investigating the protein expressions in *R. mucronata* leaves under shade regimes in relation with photosynthesis and photoprotection mechanisms by a proteomic approach.

The significance reduction in photosynthetic performance of *R. mucronata* seedlings under shade regimes, however, was not attributed to variability in chlorophyll, Ci, ΦPSII, ETR or qP. Reduction in CO_2 exchange under deep shade conditions was more due to differences in g_s, E, and carboxylation efficiency which decreased CO_2 fixation capacity of LL seedlings. HL and ML leaves sustained a better photosynthetic performance at higher leaf temperature rather than LL leaves. Furthermore, though HL seedlings achieved high P_{max}, severe symptoms of decoloring leaves degraded their value and interfered seedlings' growth. Moreover, ML tendency had similar P_{max} with HL but in lower level of g_{max} and $E_{max.}$ In order to obtain a high growth and carbon fixation capacity of *R. mucronata* seedlings, we recommend trying to achieve approximately 50% ambient light with a 50% shade net (ML treatment). This is consistent with the habitat of mangrove *R. mucronata* that common on transition zone between *Bruguiera* and *Avicennia*.

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Study on Photosynthetic Responses and Chlorophyll Fluorescence in Rhizophora mucronata seedlings under Shade Regimes

--Manuscript Draft--

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Corresponding Author:	Akihiro Nose, Prof, Ph.d Saga University SAGA, SAGA JAPAN
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Saga University
Corresponding Author's Secondary Institution:	
First Author:	Tengku Zia Ulqodry
First Author Secondary Information:	
Order of Authors:	Tengku Zia Ulqodry
	Fumiko Matsumoto
	Yosuke Okimoto, Ph.D
	Akihiro Nose, Prof, Ph.d
	Shao-Hui Zheng, Ph.D
Order of Authors Secondary Information:	
Abstract:	Seasonal gas exchange and chlorophyll fluorescence were investigated to evaluate photosynthetic performance of mangrove Rhizophora mucronata seedlings grown under full light (HL), 50% shade (ML) and 80% shade (LL) conditions. Significance increasing in SPAD which had a tight correlation with chlorophyll content indicated a strategy to adapt with excess or deficiency light intensity. HL and ML had higher photosynthetic rate (PN), stomatal conductance (gs) and transpiration rate (E) than LL leaves. ML tendency had similar maximum PN with HL but in lower level of maximum stomatal conductance (gmax) and maximum transpiration rate (Emax). We found that carboxylation efficiency significantly affected the seasonal change of photosynthetic capacity. The carboxylation efficiency of LL leaves was tendency lower than HL and ML leaves. The photosynthetic performance of R. mucronata seedlings under shade regimes, however, was not attributed to variability in chlorophyll, Ci, ΦPSII, ETR or qP but more due to differences in carboxylation efficiency, gmax, and Emax, respectively. HL and ML leaves sustained a better photosynthetic performance at higher leaf temperature rather than LL leaves, but LL sustain low susceptibility to photoinhibition. The highest non-photochemical quenching at HL leaves represented that the using of light energy probably already exceed photosynthetic capability. The findings indicate that ML treatments showed better ability to obtain a high carbon fixation capacity which consistent with the habitat of R. mucronata that common on transition zone.

LIST OF CHANGES

Page	Line	Deleted	Formatted
1	5		Introduction
1	7-10	Subject to immediate daily, monthly, and annual variation in their physical environment, mangroves have a remarkable ability to cope with stress conditions (McLeod and Salm 2006). Light, salinity, and flooding are considered as the dynamic stressors in mangrove habitat.	Subjects to daily, monthly, and annual variations in their physical environment, mangroves have a remarkable ability to survive with stress conditions (McLeod and Salm 2006). Especially light, salinity, and flooding are considered as the dynamic stressors in mangrove habitat.
1	11-13	Adaptation in shade tolerance is one of some causes of mangrove distribution patterns (Macnae 1969). Significant differences in survival were found among mangrove species, between intertidal zones and due to light level (Smith 1987).	Adaptation to shade is one of the causes of mangrove distribution patterns (Macnae 1969). Significant differences in the survival rates of the mangrove species were found depending on their intertidal positions and light exposition (Smith 1987)
1	14	ever present	ever-present
1	15-17	In the other side, Smith (1987) stated that the influence of light did not appear to be as valuable as hypothesized. Although significant light effects were found, the differences in growth and survival of seedlings grown in light and shade were small. Furthermore, the different of light requirements among mangrove species indicated light-dependent responses of photosynthetic rate (Clough 1998).	Furthermore, the different of light requirements among mangrove species indicated light-dependent responses of photosynthetic rate (Clough 1998) with different responses for each mangrove species (Kitao et al. 2003; Krauss and Allen 2003).
1	18	the group of plant which use C_3 photosynthesis	the C_3 plants
1	19	In relation with	As regards
1	20	showed	is
1	21	favorably	favourably
1	22		is considered as
1	25		in
1	27	common	commonly
1	28	insolation	insulation
2	40-41	The temperature grade is substantially higher than the actual temperature in the canopy, causing an overestimation of CO_2 emission (Okimoto et al. 2007).	The temperature grade is substantially higher than the actual temperature in the mangrove canopy (Okimoto et al. 2007)
2	42	shade	shaded
2	43-45	Conversely, what happens to a sun leaf offer any advantage when it is under low temperature?	Conversely, what happens with a leaf originally sunned, has the lowering temperature upon shading any advantage for its functioning?
2	45-46	sun and shade	sunned and shaded
2	46	seedling under ambient greenhouse temperature for 1 year	seedling for 1 year
2	48-49	will contribute to a better improving on photosynthetic capacity as estimation of mangrove growth model.	will contribute to a better improving on photosynthetic capacity as estimation of mangrove productivity.
2	55-56	created by using	done by neutral
2	56	experiment	the experiment
3	58	mid day	midday

3	58	showed	and showed
3	59-60	for LL, ML and HL treatments ranged from 345, 885 and 1728 µmol photon m^{-2} s^{-1}, respectively	was 1728, 885, and 345 µmol photon m^{-2} s^{-1} for HL, ML and LL treatments, respectively.
3	60-61		It showed that the shading level after 1 year treatment was still consistent at full sunlight, 50% and 80% shading conditions
3	61	Monthly	The monthly
3	62	measured with	recorded hourly with
3	63-65	is displayed in Fig 1	The maximum, minimum and average temperature from each day were determined, and these daily values were average over a month to get the data points displayed in Fig 1.
3	71	in the morning (08:00 h, local time) until close to mid-day (11:00 h).	sunny days from the morning (08:00 h, local time) until close to mid-day (11:00 h) only.
3	78-80		In order to minimize the temperature shock effect, the starting temperatures were different for each seasons, they were lower during cold months than hot months.
3	80	the rate of photosynthetic	the photosynthetic rate
4	88	by 0.8-s pulse	by a 0.8 s pulse
4	89	steady state value of fluorescence	steady state fluorescence
4	90	subjected	applied
4	103-104		Statistical analysis: All statistical tests were performed with Tukey HSD's test to detect differences between means. Significant differences are reported as $P < 0.05$.
4	107	had effects on	affected
4	108-109	Leaf color of plants grown under 80 % shade were dark green, while those grown under 50% shade and full sunlight were green and light green respectively (Fig 2)	Leaf colour of LL-plants were dark green, while those of ML- and HL-plants were green and light green respectively (Fig 2)
4	110	reading which had a	readings being in
4	110-111	always show the similar pattern for each months, there were HL<ML<LL respectively (Fig 3)	showed similar HL<ML<LL pattern for each months (Fig 3).
4	111-112	The lowest SPAD value of HL and ML leaves occurred on February while LL leaves happened on July 2012	HL and ML leaves showed seasonal SPAD value variation and exhibited a slight minimum around February 2012
4	113-114		The minimum SPAD value for LL leaves occurred in July 2012, but did not show significant seasonal variation.
5	129	point	points
5	130	shinning	sunny
5	130	increased	increase
5	135	saturating conditional	saturation
5	140	fit	fitted
6	147-148		The temperature responses of P_N tend to show seasonal variation while leaf temperature controlled at 30 ^{0}C rather than 25 ^{0}C.
6-7	162-169		Furthermore, maximum photosynthetic rate responses to C_i (P_{max}-C_i) that represent the capacity of leaf photosynthesis is also determined from Eq. 1 while C_i become infinity, i.e.

			$P = \frac{I}{\alpha + \beta \cdot I}$ $\frac{1}{P} = \frac{\alpha}{I} + \beta$, and while *I* become ∞ $P_{\max - Ci} = \frac{1}{\beta}$ (3) where P_{max}-C_i is maximum photosynthetic rate responses to C_i and β is second coefficients to determine the convexity of the hyperbola. Figure 8 showed that initial slope of P_N (C_i) had similar seasonal change with P_{max}-C_i.
7	168-169		Figure 8 showed that initial slope of P_N (C_i) had similar seasonal change with P_{max}-C_i.
7	169-170	initial slope during hot months were higher rather than cold months, whereas declined from August 2011 to March 2012, and went up again until July 2012.	Both of P' and P_{max}-C_i during hot months were higher than that of the cold months; actually, it declined from August 2011 to March 2012, and went up again until July 2012.
7	171-173	We also found that initial slope of LL leaves was tendency lower than HL and ML leaves	We also found that initial slope of LL leaves was slight lower than HL or ML leaves between October-February and April-July 2012.
7	174	by both temperature and shade regimes.	by pre-condition temperature mainly and shade regimes.
7	177	after 30 minutes dark-adaptation on all observation months indicate higher values during hot months compared with cold months.	measured after 30 minutes exhibited the same seasonal variations as the other photosynthetic parameters
7	180	LL showed relatively high values of ΦPSII and ETR after dark adaptation compared with HL and ML (Fig 9)	LL showed relatively high values of ΦPSII and ETR as compared with HL and ML (Fig 9).
7	183-185	The highest qP value of HL and LL occurred at September 2011 while ML ensued at July 2012 (Fig 10 a). Unexpectedly, the qP value for HL also high on February 2012,	The qP values showed a slight seasonal variation that higher during April-November than cold months (December-March) (Fig 10 a). Unexpectedly, the qP value for HL also high in February 2012,
7	187	higher compared	higher as compared
7	188	We also found that the qN values of LL leaves usually lower than other treatments during 1 year observation.	
7	190-192	Significance decreasing of Fv/Fm for HL and ML leaves mainly occurred on February and March 2012 while for LL leaves happened on July 2012 (Fig 11).	HL and ML leaves showed seasonal Fv/Fm ratio variation and exhibited a significant decreasing in February and March 2012 (Fig 11).
7	194	significance increasing	significantly increased
7	194	value	values
7	194	raising leaf size	leaf sizes
7	194		in plants
7	195	shade regimes, they were HL<ML<LL respectively	50 and 80% shading
7	195	It indicate a	These results indicate the
8	196	with excess or deficiency light intensity. The ways of	extreme light intensities:
8	196		their

8	197	; conversely,	; in contrast
8	197		their
8	197	raising	rising their
8	199	escalate their	increase their
8	199-200	and increasing	to optimize
8	203	However, we	We have also
8	203	decoloring	decolouring
8	204		must have been
8	205	The decoloring can be occurred	Decolouring may occur
9	209	decoloring	decolouring
9	209-211	with no significance chlorophyll content of mangrove *Kandelia candel* grown at 15 and 30 ^{0}C (Kao et al. 2004)	with no significance decreasing chlorophyll content of mangrove *Kandelia candel* grown either at 30 or 15 ^{0}C (Kao et al. 2004).
8	211-212	Although LL got significance reduction SPAD value on July 2012 but still higher than HL and ML on the same month (Fig.3).	Altohugh LL exhibited a significantly reduced SPAD value in July, but this value was still higher than those of the HL and LL leaves in the same period (Fig.3).
8	212-214	We suggested that this is not decoloring symptom but more than as LL protection mechanism to adapt with the incident high radiation on July 2012.	We suggest that the slight minimum SPAD value of LL leaves in July 2012 as LL protection mechanism to adapt with the incident high radiation.
9	238	80% shaded and unshaded conditional	LL and HL conditions
9	240	from understory shade	from shade
9	241-242	while compared with	while compared with than those
9	242-244	This result also elucidate the zonation pattern of *R. mucronata* that common found between zonation of *Avicennia* and *Bruguiera* (White et al. 1989 ; Whitten et al. 2000).	This finding corroborates the idea of Kitao et al (2003), who suggested that within intermediate gap-phase species, *Rhizophora* prefers more sun-lit sites than *Bruguiera*.
9	244	that showed difference	showing different
9	245	between hot months	in the hot
9	245	with cold months (December-March)	and in the cold (December-March) months
9	253	the latter partly reported	some latter reports indicate
10	256	depended on	which was depending on the
10	256	During	At
10	256	temperature	temperatures
10	257-258	obtained at leaf temperature between 29 and 34 ^{0}C, and decrease at 23-29 ^{0}C at others month	between 29-34 ^{0}C leaf temperatures, but at lower (23-29 ^{0}C) leaf temperatures in the other months
10	259	rather than	as compared to
10	260	for plants	for plant
10	260	highly	strongly
10	261	the temperature under which the plants had been grown	their growth-temperature
10	261-262	Deep shade area might create lower temperature rather than open area	The temperature is lower in deep-shade areas than the sun-exposed ones
10	262	had	exhibited
10	263	temperature	temperatures
10	264-265	the simplicity	the stomata responses
10	270-275		Furthermore, maximum photosynthetic rate responses to C_i is beneficial to indicate the capacity or potential of leaf photosynthesis. As shown in Fig 8, the similar seasonal pattern of P' and P_{max}-C_i suggested that the potential photosynthesis of *R. mucronata* leaves was strongly affected by carboxylation efficiency. Both of them were

			higher on hot months compared with on cold months. In contrast to Sage and Reid (1994) that reported the initial slope P_N (C_i) is slightly affected by temperature, we found that seasonal variation of temperature significantly affect P' and P_{max}.C_i.
10	281-282	long term	long-term
10	282	to	of
12	320	decoloring	decolouring
12-13	336-342		Acclimation to various light intensities may have an influence not only on photosynthesis processes but also several physiological and biochemical processes, including acclimation mechanisms, which are not directly related to photosynthesis. Gray et al (1997) reported that light as the fundamental energy source for all photoautotrophs affected PSII excitation pressure appear to extend beyond photosynthetic acclimation, to influence expression of a nuclear gene involved in low temperature acclimation. Furthermore, the expression levels of several photosynthesis- and hormonal-related genes were significantly affected by the light intensity (Majláth et al 2012).
13	345		**Conclusions**
13	346-355	The significance reduction in photosynthetic performance of *R. mucronata* seedlings under shade regimes, however, was not attributed to variability in chlorophyll, Ci, ΦPSII, ETR or qP. Reduction in CO_2 exchange under deep shade conditions was more due to differences in g_s, E, and carboxylation efficiency which decreased CO_2 fixation capacity of LL seedlings. HL and ML leaves sustained a better photosynthetic performance at higher leaf temperature rather than LL leaves. Furthermore, though HL seedlings achieved high P_{max}, severe symptoms of decoloring leaves degraded their value and interfered seedlings' growth. Moreover, ML tendency had similar P_{max} with HL but in lower level of g_{max} and $E_{max.}$ In order to obtain a high growth and carbon fixation capacity of *R. mucronata* seedlings, we recommend trying to achieve approximately 50% ambient light with a 50% shade net (ML treatment). This is consistent with the habitat of mangrove *R. mucronata* that common on transition zone between *Bruguiera* and *Avicennia*.	The results confirm that the seasonal change of photosynthetic capacity was affected strongly by carboxylation efficiency. The photosynthetic performance of *R. mucronata* seedlings under shade regimes, however, was not attributed to variability in chlorophyll, Ci, ΦPSII, ETR or qP but more due to differences in carboxylation efficiency, g_{max}, and E_{max}, respectively. HL and ML leaves sustained a better photosynthetic performance at higher leaf temperature rather than LL leaves, but LL sustain low susceptibility to photoinhibition. Our findings indicate that seedling grown under moderate shade condition showed better ability to obtain a high carbon fixation capacity which consistent with the habitat of *R. mucronata* that common on transition zone. This result is important to elucidate the zonation pattern of mangrove and also to clarify the suitable shading level during nurse phase of *R. mucronata* in reforestation and cultivation activity.
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Keywords. Chlorophyll Fluorescence, Mangrove, Photoinhibition, Photosynthesis, *Rhizophora mucronata*, Shade tolerance

Abbreviations.

C_i	Intercellular CO_2 concentration
E	Transpiration rate
E_{max}	Maximum transpiration rate
ETR	Electron transport rate
Fv/Fm	Ratio of variable to maximum chlorophyll fluorescence
g_{max}	Maximum stomatal conductance
g_s	Stomatal conductance
PAR	photosynthetically active radiation
P_{max}	maximum photosynthetic rate
P_N	Net photosynthetic rate
PSII	Photosystem II
qN	Non-photochemical quenching
qP	Photochemical quenching
Vpdl	Vapour pressure deficit between the leaf and air
ΦPSII	Quantum yield of Photosystem II

Introduction

Mangroves represent an important coastal ecosystem in the tropic area because of their high productivity and adaptation ability under various abiotic stresses. Subjects to daily, monthly, and annual variations in their physical environment, mangroves have a remarkable ability to survive with stress conditions (McLeod and Salm 2006). Especially light, salinity, and flooding are considered as the dynamic stressors in mangrove habitat.

Adaptation to shade is one of the causes of mangrove distribution patterns (Macnae 1969). Significant differences in the survival rates of the mangrove species were found depending on their intertidal positions and light exposition (Smith 1987). One hypothesis claimed that shade intolerance of mangrove seedlings was an additional stress on the ever-present stressor, salinity (Janzen 1985). Furthermore, the different of light requirements among mangrove species indicated light-dependent responses of photosynthetic rate (Clough 1998) with different responses for each mangrove species (Kitao et al. 2003; Krauss and Allen 2003).

Mangroves belong to the C_3 plants that might show differences in photosynthetic capacity and sensitivity to environmental conditions for different species (Ball 1986). As regards light competition, gas exchange and chlorophyll fluorescence characteristics of mangrove *Avicennia marina* is typical of sun leaves (Ball and Crithcley 1982). On the other hand, *Bruguiera sexangula* responded favourably to short burst of sunlight at low light level and is considered as relatively shade tolerant species (Krauss and Allen 2003).

Rhizophora mucronata Lamk, “the intermediate gap-phase mangrove species”, is found worldwide from East Africa and India through Asia as well as Indonesia to the western Pacific, in wet tropical regions of Australia and in Mozambique and South Africa (Hoppe-Speer et al. 2011). In Indonesia, *R. mucronata* commonly found between zonation of *Avicennia* and *Bruguiera* (White et al. 1989 ; Whitten et al. 2000) that occupies a gradient from low intertidal swamp margins with high insulation, to shaded sites at high water. *R. mucronata* had a role as main plant in the reforested thinned site in tropical coastal

area (Srivastava et al. 1988) and produced more leaf litter than the reforested unthinned and natural sites (Wang'ondu and Virginia 2010). While thinning activity contribute on shading conditions, information of seedlings adaptive capacity to shade regimes in relation to photosynthetic performances is essential to clarify both the mangrove zonation pattern and the growth model of *R. mucronata* in the restoration area.

Light or shade regimes were considered to affect not only photosynthetic rate but also chlorophyll fluorescence. Exposure to excess irradiance can lead to photoinhibition, which is characterized by a light-dependent reduction in the fundamental quantum yield of photosynthesis and a loss of photosystem II (PSII) activity (Osmond 1994). So far, there is no specific information about chlorophyll fluorescence of *R. mucronata* seedlings under shade regimes.

The contrasting low- and high-shading areas will create varying combinations of light and temperature also. The temperature grade is substantially higher than the actual temperature in the mangrove canopy (Okimoto et al. 2007). Ong et al (1995) reported that the temperature on the top of the mangrove canopy was about 10 ^{0}C higher than at the ground surface. If a shaded leaf becomes exposed to full sunlight, does its temperature exceed the optimum for photosynthesis? Conversely, what happens with a leaf originally sunned, has the lowering temperature upon shading any advantage for its functioning? To answer such questions, we also investigated the photosynthetic responses of sunned and shaded leaves of *R. mucronata* seedling for 1 year, while the temperature is different at each months.

Finally, seasonal information of photosynthetic rate and chlorophyll fluorescence in *R. mucronata* seedlings under shade regimes will contribute to a better improving on photosynthetic capacity as estimation of mangrove productivity.

Materials and Methods

Plant materials and growth conditions: Propagules of *R. mucronata* were collected from Galang Island (0° 45' N, 104° 15' E) in Batam District, Indonesia. Propagules were planted in the greenhouse with heating system at the Laboratory of Tropical Crop Improvement, Faculty of Agriculture, Saga University, Japan (33° 14' N, 130° 17' E) on June 2010. After five months, seedlings with 3-4 pairs of leaves were grown under full sunlight (HL), 50% shading (ML) and 80% shading (LL). Shade treatments were done by neutral density black nylon netting. During the experiment, seedlings were watered to ensure that drought did not confound experimental results.

Light intensities were measured on midday at July 20, 2012, a sunny cloudless day, and showed that the actual photosynthetically active radiation (PAR) was 1728, 885, and 345 µmol photon m^{-2} s^{-1} for HL, ML and LL treatments, respectively. It showed that the shading level after 1 year treatment was still consistent at full sunlight, 50% and 80% shading conditions. The monthly variation of air temperature in the greenhouse from August 2011 to July 2012, recorded hourly with a portable Thermo Recorder equipped with an external thermosensor (TR-50C, T and D co. Ltd., Nagano, Japan). The maximum, minimum and average temperature from each day were determined, and these daily values were average over a month to get the data points displayed in Fig 1.

Leaf Gas Exchange: The responses of mangrove seedling for leaf gas exchange to shade treatments were evaluated for 1 year from August 2011 to July 2012, beginning after seedlings had been exposed to their shading treatments for 8 months. Net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were measured with a portable open-flow gas exchange system (LI-6400, Li-COR, Lincoln, NE, USA). Measurements were made at fully expanded leaves in sunny days from the morning (08:00 h, local time) until close to mid-day (11:00 h) only.

Photosynthetic rate under shade regimes was evaluated in relation to light intensity and temperature. In relation to light intensity, PAR value on leaf surfaces was automatically maintained in decreasing order from 1000 to 0 µmol m^{-2} s^{-1} (1000, 500, 250, 100, 50, 0 µmol m^{-2} s^{-1}). During the measurements, leaf temperature was controlled at 30 ^{0}C, vapour pressure deficit between the leaf and air (VpdL) was 1.7 $\pm$ 0.3 kPa, and CO_2 input was 370 µmol mol^{-1}. The effect of leaf temperature on photosynthetic rate was measured from 20 to 38 ^{0}C under PAR, VpdL and CO_2 input were 1000 µmol m^{-2} s^{-1}, 1.7 $\pm$ 0.3 kPa, and 370 µmol mol^{-1}, respectively. In order to minimize the temperature shock effect, the starting temperatures were different for each seasons, they were lower during cold months than hot months. Furthermore, the quantifying the photosynthetic rate as a function of C_i was done by changing the CO_2 concentration at the leaf surface from 0 to 1000 µmol mol^{-1}, under PAR 1000 µmol m^{-2} s^{-1} and leaf temperature 30 ^{0}C.

Chlorophyll Fluorescence: Leaf chlorophyll fluorescence was measured with a modulated chlorophyll fluorometer (OS5-FL, OPTI-SCIENCES, USA) between 08:00 h and 11:00 h, on the same leaves used for gas exchange analysis. The fluorescence parameters were obtained under both dark-adapted fluorescence and yield of energy conversion as described by Genty et al (1989). In leaves submitted to

darkness, readings were taken after 30 minutes dark adaptation using a leaf clip. Minimum fluorescence (Fo) was determined by a weak red light and maximum fluorescence (Fm) was induced by a 0.8 s pulse of 2000 μmol m^{-2} s^{-1} PAR. The steady state fluorescence (Fs) was recorded and a second saturating pulse was applied to determine the maximum light-adapted fluorescence (Fm'). A 685 nm light source equipped with OS5-FL was used for the illumination of leaf as actinic light. The actinic light was removed then the minimum fluorescence level in the light-adapted state (Fo') was determined after 10 s of far red illumination. The following chlorophyll fluorescence parameters were calculated according to Genty et al (1989) and Maxwell and Johnson (2000): quantum yield of Photosystem II, ΦPSII = (Fm'-Fs)/Fm'; maximum quantum efficiency of fluorescence PSII, Fv/Fm = (Fm-Fo)/Fm; photochemical quenching coefficient, qP = (Fm'-Fs) / (Fm'-Fo'); non-photochemical quenching, qN = (Fm-Fm') / (Fm-Fo'); and electron transport rate, ETR = ΦPSII x PAR x 0.5 x 0.84. PAR corresponds to the flux density of incident photosynthetically active radiation, 0.5 was as a factor that accounts for the portioning of energy between PSII and PSI, and 0.84 was assumed from an average of 84% of the incident light were absorbed by the leaf.

SPAD measurement: SPAD reading as representative of relative chlorophyll content was measured by using SPAD-Chlorophyll meter (SPAD 502, Minolta, Osaka, Japan).

Statistical analysis: All statistical tests were performed with Tukey HSD's test to detect differences between means. Significant differences are reported as $P < 0.05$.

Results

Leaf morphology and SPAD value.

Shade treatments affected *R. mucronata* leaf morphology. LL leaves were larger than HL and ML leaves. Leaf colour of LL-plants were dark green, while those of ML- and HL-plants were green and light green respectively (Fig 2).

SPAD readings being in tight correlation with chlorophyll content (Markwell et al. 1995) showed similar HL<ML<LL pattern for each months (Fig 3). HL and ML leaves showed seasonal SPAD value variation and exhibited a slight minimum around February 2012. Furthermore, decreasing SPAD value of HL leaves also occurred in July 2012. The minimum SPAD value for LL leaves occurred in July 2012, but did not show significant seasonal variation.

Effects of light intensity on P_N, g_s, E, and Ci.

Variation of P_N responses to light intensity at 30 ^{0}C of leaf temperature showed almost similar trends for all three treatments, increased simultaneously with PAR escalation until reaching their saturation point (Fig 4).

The light responses of P_N, g_s and E were determined using the rectangular hyperbola model (Okimoto et al. 2008; Table 1):

$$P = \frac{I}{\alpha + \beta \cdot I} \quad (1)$$

where P is P_N of individual leaves at light intensity of I (μmol photons m^{-2} s^{-1}), then α and β are coefficients to determine the convexity of the hyperbola. When used to model of conductance and transpiration responses, P was substituted to represent the g_s and E values in Eq.1. HL and ML had higher P_N, g_s and E than LL leaves while PAR increasing.

Equation 1 was used to determine maximum photosynthetic rate (P_{max}), maximum stomatal conductance (g_{max}), and maximum transpiration rate (E_{max}) at light saturation conditional (Table 1). The light saturation points of all treatments were commonly at PAR level around 1000 μmol photons m^{-2} s^{-1}. P_N, g_s and E responses to light during hot and sunny months (June-September) tended to increase rapidly up to PAR 100 μmol m^{-2} s^{-1}, had high values and wide gap value between shading treatments at saturation point. In the other side, during cold months (December-March) they were characterized with rapid increasing up to PAR about 250 μmol m^{-2} s^{-1}, low values and no significance difference at saturation point (Fig 4).

Under light saturation, P_{max} showed a positive correlation with g_{max} and E_{max} (Fig 5). The highest values of g_{max} and E_{max} showed similar trends, there were LL<ML<HL respectively. Lower rates of g_{max} and E_{max} for LL leaves probably restricted P_{max}. We found that although the highest value of g_{max} and E_{max} of ML were lower than HL, but their highest P_{max} value were tendency similar.

Effect of temperature on photosynthesis.

The quadratic curves were fitted to describe the temperature responses of P_N (Fig 6). The results showed that relationship between P_{max} and leaf temperature indicated a broad peak for difference season. During mid-high temperature months between August-November 2011 and May-July 2012, P_{max} was obtained at leaf temperature between 29-34 ^{0}C, and decrease at 23-29 ^{0}C on cold months (December

2011-April 2012). P_{max} for the temperature responses of HL (14.9 μmol m^{-2} s^{-1}) and LL (12.0 μmol m^{-2} s^{-1}) occurred on September 2011 at leaf temperature 32 ^{0}C, while ML (13.8 μmol m^{-2} s^{-1}) ensued on July 2012 at 33 ^{0}C.

The temperature responses of P_N tend to show seasonal variation while leaf temperature controlled at 30 ^{0}C rather than 25 ^{0}C. We also found that LL leaves sustained a better photosynthetic performance at leaf temperature 25 ^{0}C than HL and ML leaves. In contrast, at leaf temperature 30 ^{0}C, P_N of HL and ML leaves was higher generally than LL leaves (Fig 7).

Effect of C_i on photosynthesis.

The carboxylation efficiency relating with Rubisco activity can be estimated as the initial slope of the response P_N to C_i (Ku and Edwards 1977; Sage and Reid 1994). The initial slope of P_N (C_i) curve is calculated and derived from Eq. 1 while Ci tend to zero, i.e.

$$P = \frac{I}{\alpha + \beta \cdot I}$$

$$P' = \frac{\alpha + \beta \cdot I - \beta \cdot I}{(\alpha + \beta \cdot I)^2}$$, and while *I* toward zero

$$P' = \frac{\alpha}{\alpha^2}$$

$$P' = \frac{1}{\alpha} \quad (2)$$

where P', *I* and α are initial slope of P_N (C_i) curve, intercellular CO_2 concentration and first coefficients to determine the convexity of the hyperbola, respectively. The carboxylation efficiency implied increasing in photosynthetic rate achieved per unit increasing in CO_2 at the site of CO_2 fixation. Furthermore, maximum photosynthetic rate responses to C_i (P_{max}-C_i) that represent the capacity of leaf photosynthesis is also determined from Eq. 1 while C_i become infinity, i.e.

$$P = \frac{I}{\alpha + \beta \cdot I}$$

$$\frac{1}{P} = \frac{\alpha}{I} + \beta$$, and while *I* become ∞

$$P_{\max - Ci} = \frac{1}{\beta} \quad (3)$$

where P_{max}-C_i is maximum photosynthetic rate responses to C_i and β is second coefficients to determine the convexity of the hyperbola. Figure 8 showed that initial slope of P_N (C_i) had similar seasonal change with P_{max}-C_i. Both of P' and P_{max}-C_i during hot months were higher than that of the cold months; actually, it declined from August 2011 to March 2012, and went up again until July 2012. It suggested that seasonal change of leaf photosynthetic capacity was controlled by carboxylation efficiency. We also found that initial slope of LL leaves was slight lower than HL or ML leaves between October-February and April-July 2012. This result indicated that carboxylation efficiency of *R. mucronata* leaves were influenced by pre-condition temperature mainly and shade regimes.

Chlorophyll fluorescence.

The seasonal variation of quantum yield of PSII (ΦPSII) and electron transport rate (ETR) measured after 30 minutes exhibited the same seasonal variations as the other photosynthetic parameters. The ΦPSII and ETR decreased from August 2011 to February 2012, then increased from March until July 2012. Their lowest values occurred on February 2012. During cold months (December 2011-March 2012), LL showed relatively high values of ΦPSII and ETR as compared with HL and ML (Fig 9).

Photochemical quenching (qP) is a ratio of light energy used in the transfer of photochemical electrons to total light energy captured by antenna pigment and non-photochemical quenching (qN) reflects a ratio of light energy consumed by heat to the total light energy (Zhou et al. 2010). The qP values showed a slight seasonal variation that higher during April-November than cold months (December-March) (Fig 10 A). Unexpectedly, the qP value for HL also high in February 2012, whereas the P_N and SPAD value were low (Table 1, Fig 3). Furthermore, in September 2011 and between December 2011-February 2012, qN values of HL leaves were higher as compared with other treatments (Fig 10 B).

A reduction in the ratio of variable to maximum chlorophyll fluorescence (Fv/Fm) can be used as an indication of photoinhibition (Björkman and Demmig 1987; Robakowski 2005). HL and ML leaves showed seasonal Fv/Fm ratio variation and exhibited a significant decreasing in February and March 2012 (Fig 11).

Discussion

The results showed significantly increased SPAD values ($P < 0.05$) and leaf sizes while in plants exposed to 50 and 80% shading (Fig 2 and 3). These results indicate the strategy of *R. mucronata*

seedlings to adapt extreme light intensities: HL seedlings decreased their light absorption by reducing chlorophyll content and leaf area; in contrast, LL seedlings increased their light absorption by rising their leaf area and chlorophyll content. Previous studies have shown that plants grown under shaded conditions were noted to increase their pigment density per unit leaf area (Wittmann et al. 2001, Xu et al. 2009), to optimize their height, leaf area, crown extension and leaf arrangement to get the best use of light (Paquette et al. 2007, Huang et al. 2011). When growing in a high-light environment, avoidance of light absorption, e.g. through low chlorophyll contents, played a crucial role in protecting the photosynthetic apparatus of leaves (Adams et al. 2004). We have also found decolouring symptom with lower SPAD value of HL and ML leaves that must have been caused mainly by low temperature in February 2012. Decolouring may occur as a consequence of the combined effects of high incident PAR and low temperature (Close et al. 1999). Especially for HL and ML leaves of *R. mucronata*, these results were in agreement with Kao et al (2004) findings which showed that leaves of mangrove *Avicennia marina* during low temperature at 15 ^{0}C had a greater reduction in chlorophyll content rather than 30 ^{0}C. In the other side, LL leaves had not decolouring symptom during low temperature, it was almost similar with no significance decreasing chlorophyll content of mangrove *Kandelia candel* grown either at 30 or 15 ^{0}C (Kao et al. 2004). Altohugh LL exhibited a significantly reduced SPAD value in July, but this value was still higher than those of the HL and LL leaves in the same period (Fig.3). We suggest that the slight minimum SPAD value of LL leaves in July 2012 as LL protection mechanism to adapt with the incident high radiation. The reduction of photosynthetic pigments could be seen as a protection mechanism as it would mitigate the capacity of the leaf to absorb incident radiation and therefore demote the amount of excess excitation energy that has to be dissipated (Burritt and Mackenzie 2003).

Significant increases in total chlorophyll lead raising in CO_2 exchange were due to increased photosynthetic rate (Evans 1989), as shown in mangrove *A. marina* and *Hibiscus tiliaceus* (Naido et al. 2002). However, this study has been unable to demonstrate that higher total chlorophyll had high P_N in *R. mucronata* seedlings under shade regimes. The contrary result showed that HL and ML had higher P_N than LL leaves while PAR increasing (Fig 4). We found that under light saturating conditional, g_{max} and E_{max} showed similar trends, there were LL<ML<HL respectively (Fig 5, Table 1). It described that the P_{max} of *R. mucronata* seedlings were more influenced by g_{max} and E_{max} rather than chlorophyll content. The circulation of CO_2 is determined by stomatal density, size, and conductance (Xuan et al. 2011), and

among of those factors, stomatal conductance is the most prominent (Putra et al. 2012). Cheeseman et al (1997) found that the relationship between net CO_2 assimilation and g_s in mangrove *Rhizophora stylosa* was significant and positive while measured under intermediate temperature and high light. Lower rates of g_{max} for LL leaves probably restricted the maximum photosynthetic rate, that similarly as shown at "the shade tolerant mangrove species", *Bruguiera sexangula* (Krauss and Allen 2003). High stomatal conductance was followed by increased transpiration rate. The positive relationships between P_N, g_s and E were also found at mangroves seedlings of *R. stylosa* grown under light levels (Kitaya et al. 2002). Moreover, ability of ML leaves to achieve high P_{max} in lower g_{max} and E_{max} compared with HL leaves, indicate ML effectiveness and also chance to conserve water in better level. It will be useful while ML seedlings adapt with saline conditional.

We found that the light saturation point of all treatments were commonly at PAR level around 1000 μmol photons m^{-2} s^{-1}. These results were higher than mangrove *B. sexangula* and similar with *A. marina.* The finding of Krauss and Allen (2003) estimated that light saturation point of *B. sexangula* seedlings usually below 500 μmol photons m^{-2} s^{-1} under both LL and HL conditions. The assimilation rates of *A. marina*, "the sunlit mangrove species" became light saturated at approximately 1000 μmol photons m^{-2} s^{-1} in leaves from shade condition and high light regime (Ball and Critchley 1982). It can therefore be assumed that *R. mucronata* leaves are more a sunny leaf type while compared with than those of *B. sexangula*. This finding corroborates the idea of Kitao et al (2003), who suggested that within intermediate gap-phase species, *Rhizophora* prefers more sun-lit sites than *Bruguiera*.

Our finding showing different characteristics of P_N responses of *R. mucronata* leaves to light intensity (Fig 4) in the hot (June-September), and in the cold (December-March) months emphasized the role of temperature for mangrove seedling growth and photosynthetic performances. Low temperature clearly modified the passage of light response curves on cold months compared with hot months.

Photosynthesis of mangroves has been indicated to be highly sensitive to leaf temperature (Andrews et al. 1984; Ball et al. 1988). In view of the ecological distribution of plants, it was necessary to explain the temperature response curve of photosynthesis (Agata et al. 1985), and also could improve the accuracy of estimation of CO_2 fixation capacity by mangrove (Okimoto et al. 2007). Moore et al (1973) reported that P_{max} of mangrove *Rhizophora* and *Laguncularia* was obtained at leaf temperature near or below 25 ^{0}C. In contrast, some latter reports indicate that the relationship between the net

photosynthetic rate and leaf temperature indicated a wide peak between 29 and 34 ^{0}C (Okimoto et al. 2007). Our finding showed that relationship between P_{max} and leaf temperature indicated a broad peak, which was depending on the pre-condition temperature. At high pre-condition temperatures between August-November 2011 and May-July 2012, P_{max} was obtained between 29-34 ^{0}C leaf temperatures, but at lower (23-29 ^{0}C) leaf temperatures in the other months (Fig 6). We also found that LL leaves sustained a better photosynthetic performance at lower leaf temperature as compared to HL and ML leaves (Fig 7). Some studies have found that the optimum temperature for plant photosynthesis depended strongly on their growth-temperature (Sawada and Miyachi 1974; Kao et al. 2004). The temperature is lower in deep-shade areas than the sun-exposed ones, thus, LL seedlings exhibited better photosynthetic performance at lower temperatures.

Sharkey (1985) pointed out that the rates of photosynthesis were a function of both the stomata responses to allow carbon dioxide to penetrate the leaf and the biochemical capacity to fix CO_2. Change in the shape of the P_N (C_i) curve was not only beneficial to indicate variability in the capacity for photosynthesis, but also elucidate which regions of photosynthetic biochemistry are sensitive to environment (Ball 1986). Initial slope of the response of P_N to C_i could be correlated to *in vivo* assessment of biochemical components of leaf photosynthesis, such as ribulose-biphosphate carboxylase (rubisco) activity (Caemmerer and Farquhar 1981). Furthermore, maximum photosynthetic rate responses to C_i is beneficial to indicate the capacity or potential of leaf photosynthesis. As shown in Fig 8, the similar seasonal pattern of P' and P_{max}-C_i suggested that the potential photosynthesis of *R. mucronata* leaves was strongly affected by carboxylation efficiency. Both of them were higher on hot months compared with on cold months. In contrast to Sage and Reid (1994) that reported the initial slope P_N (C_i) is slightly affected by temperature, we found that seasonal variation of temperature significantly affect P' and P_{max}-C_i. This result was in agreement with Campbell et al (2005) findings which showed increasing temperature increased the initial slope and the maximum rate of assimilation. During hot months, the low initial slope of LL leaves also supported the lower P_N and P_{max}-C_i of LL leaves compared with HL and ML leaves. This result suggested that the carboxylation efficiency of *R. mucronata* leaves was also influenced by shade regimes. Sage and Reid (1994) reported that the changes in the content of the major photosynthetic constituent (PSII content, ATP synthase, rubisco) occur with the greatest rate of adjustment after long-term acclimation to light regimes.

Φ PSII is the proportion of absorbed energy being used in photochemistry (Maxwell and Johnson 2000) that represents the efficiency of energy conversion of open PSII (Schreiber et al. 1994), and ETR represents the relative quantity of electron passing trough PSII during steady-state photosynthesis (Tezara et al. 2003). The reduction of ΦPSII and ETR for all treatments during cold months (Fig 9) were caused mainly by low temperature. Lowering the temperature generally reduces metabolic rates and can therefore limit the sinks for the absorbed excitation energy, particularly CO_2 fixation (Alam et al. 2005). A reduction in chlorophyll fluorescence in response to low temperature has also been observed in mangrove *K. candel* and *A. marina* (Kao et al. 2004). Furthermore, the combination of low temperature-high light intensity conditional during cold months might accelerate the damage to the photosynthetic apparatus (Alves et al. 2002). However, we also found that during cold months (December 2011-March 2012), LL showed relatively high values of ΦPSII and ETR after dark adaptation compared with HL and ML (Fig 10). This finding suggest that the adaptation of LL leaves in dark conditional that characterized with lower temperature rather than grown under light was helpful to protect PSII centre while exposed on low temperature.

The high qP values for all treatments during hot months are useful to sustain the high photochemical capacity. The similar patterns of the highest qP and P_{max} value for each treatments that occurred on same months (Fig 10a and Table 1) demonstrate the contribution of qP in order to P_{max} achievement level. The response of qP represented the openness of PSII centres (Kitao et al. 2003) and high qP was beneficial for the separation of electric charge in reaction centre (Dai et al. 2009). Furthermore, the high qP value of HL leaves on February 2012 whereas the P_N was low indicate abnormal conditional because of photodamage. Although the mechanism is not clear, during low temperature in cold months, it was possible that photochemical quenching was not affected by temperature. Normally, a higher in P_N resulted a higher qP in plants (Kao and Tsai 1999).

Moreover, the high qN value of HL leaves on February-March 2012 (Fig 10 b) represented that the using of light energy probably already exceed photosynthetic capability and also level of heat dissipation. qN reflects the amount of energy dissipated by non-photochemical quenching by plants (Liu et al. 2007). While photosynthesis is incapable of using all of the energy absorbed by light-harvesting complexes (Bajkan et al. 2012), the absorbed light energy not utilized in photochemistry is often dissipated thermally

(Martin et al. 2010). Furthermore, heat dissipation level that too high might cause "chlorotic" at leaves. It was similar with phenomena of the lowest SPAD value of HL leaves on February-March 2012 (Fig 3).

The regular value 0.75 - 0.85 of Fv/Fm ratios have been considered normal for unstressed plants (Hunt 2003), and decline of Fv/Fm under 0.75 could indicate a disturbance in or damage to the photosynthetic apparatus that due to photoinhibition (Litchtenthaler et al. 2005). HL & ML got photoinhibition on February and March 2012 (Fig 11), probably was caused mainly by low temperature. Photosynthesis is inhibited by low temperature, in part as an impact of reversible or reversible damage to photosynthetic structures (Robakowski 2005). The combination of low temperature and high light may affect leaf membranes and destruct the photosynthetic apparatus of higher plants (Krause 1994). Furthermore, chronic photoinhibition of HL and ML leaves might cause decolouring of photosynthetic pigments such as chlorophyll and carotenoids (Powles 1984; Takahashi et al. 2002).

In contrast with some studies that reported photoinhibition tend occurred when shade-adapted plants were exposed to high-light stress (Khan et al. 2000, Xu et al. 2009), we found that LL sustain low susceptibility photoinhibition. In this study, although Fv/Fm of LL leaves decline during cold months and shinning months, but the values were higher than 0.75 (Fig 12) and also never show chronic photoinhibition level. LL seedlings might have the ability to maintain photosynthetic activity in response to low temperature, non-freezing temperature, because of their protection mechanisms. The response of plants grown in darkness to low temperature had little effect on the PSII complex compared with under light (Alves et al. 2002). Furthermore, we suggested that the decreasing Fv/Fm of LL leaves during shinning months July 2012 simultaneously with reducing of SPAD value (Fig 3) as a mitigation strategy of the leaf to absorb incident radiation and therefore demote the quantity of excess excitation energy that has to be dissipated. Although reducing of SPAD value occurred on July 2012, but the photosynthetic performance of LL seedling was not decline (Fig 4). However, this result was also in agreement with Pompelli et al (2010) and Huang et al (2011) findings which showed that photoinhibition was not found in plants grown in shade area.

Acclimation to various light intensities may have an influence not only on photosynthesis processes but also several physiological and biochemical processes, including acclimation mechanisms, which are not directly related to photosynthesis. Gray et al (1997) reported that light as the fundamental energy source for all photoautotrophs affected PSII excitation pressure appear to extend beyond

photosynthetic acclimation, to influence expression of a nuclear gene involved in low temperature acclimation. Furthermore, the expression levels of several photosynthesis- and hormonal-related genes were significantly affected by the light intensity (Majláth et al 2012). Currently, we are investigating the protein expressions in *R. mucronata* leaves under shade regimes in relation with photosynthesis and photoprotection mechanisms by a proteomic approach.

Conclusions

The results confirm that the seasonal change of photosynthetic capacity was affected strongly by carboxylation efficiency. The photosynthetic performance of *R. mucronata* seedlings under shade regimes, however, was not attributed to variability in chlorophyll, Ci, ΦPSII, ETR or qP but more due to differences in carboxylation efficiency, g_{max}, and E_{max}, respectively. HL and ML leaves sustained a better photosynthetic performance at higher leaf temperature rather than LL leaves, but LL sustain low susceptibility to photoinhibition. Our findings indicate that seedling grown under moderate shade condition showed better ability to obtain a high carbon fixation capacity which consistent with the habitat of *R. mucronata* that common on transition zone. This result is important to elucidate the zonation pattern of mangrove and also to clarify the suitable shading level during nurse phase of *R. mucronata* in reforestation and cultivation activity.

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Fig. 1 Mean monthly, mean monthly minimum, and mean monthly maximum of greenhouse air temperature during 1 year experiment. Values are means ± SD (n=number of days in each months). Especially during cold months (December 2011-March 2012), the minimum greenhouse temperature was arranged more than 10 ^{0}C.

Fig. 2 Leaves of *R. mucronata* from the various shade treatments, (a) full sunlight (b) 50% shade (c) 80% shade. They were collected on September 16, 2012.

Fig. 3 SPAD value in leaves of *R. mucronata* grown under full sunlight (HL), 50% shade (ML), and 80% shade (LL) conditions. Values are means ± SD (n=3-4 plants). Means in the same month, followed by different letters indicated significant differences between shade regimes ($P<0.05$; Tukey HSD's test)

Fig. 4 Response of net photosynthetic rate (P_N) to increasing photosynthetically active radiation (PAR) in the leaves of *R. mucronata* seedlings grown under full sunlight (HL), 50% shade (ML) and 80% shade (LL) conditions. They were measure at leaves temperature 30 ^{0}C. Values are means ± SD (n=3-4 plants)

Fig. 5 Maximum photosynthetic rate (P_{max}) as a function of (a) maximum stomatal conductance (g_{max}) and (b) maximum transpiration rate (E_{max}) for *R. mucronata* seedlings grown under full sunlight (*diamonds* and *solid lines*), 50% shade (*squares* and *dash lines*) and 80% shade (*triangles* and *dotted lines*). Data plotted from monthly value of P_{max}, G_{max} and E_{max} at PAR 1000 μmol photon m^{-2} s^{-1} and leaf temperature 30 ^{0}C

Fig. 6 Response of net photosynthetic rate (P_N) to increasing leaf temperature *R. mucronata* seedlings grown under full sunlight (HL), 50% shade (ML) and 80% shade (LL) conditions. They were measure at leaves temperature 30 ^{0}C. Values are means ± SD (n=3-4 plants)

Fig. 7 Net photosynthetic rate (P_N) of *R. mucronata* seedlings grown under full sunlight (HL), 50% shade (ML) and 80% shade (LL) at (a) leaf temperature 25 ^{0}C and (b) 30 ^{0}C. Values are means ± SD (n=3-4 plants)

Fig. 8 Monthly pattern of initial slope (P') and maximum photosynthetic rate responses to C_i (P_{max}-C_i) of *R. mucronata* seedlings grown under full sunlight (HL), 50% shade (ML) and 80% shade (LL). They were measure at leaves temperature 30 ^{0}C PAR 1000 μmol photon m^{-2} s^{-1}. The values of P' and P_{max}-C_i were calculated with Eq.2 and Eq.3, respectively

Fig. 9 The quantum yield of PS II (ΦPSII) and electron transport rate (ETR) after 30 minutes-dark adaptation at leaves of *R. mucronata* seedlings grown under full sunlight (HL), 50% shade (ML) and 80% shade (LL) conditions. Values are means + SD (n=3-4 plants). Means in the same month, followed by different letters indicated significant differences between shade regimes ($P<0.05$; Tukey HSD's test)

Fig. 10 Comparison of (a) photochemical quenching (qP) and (b) non-photochemical quenching (qN) for leaves of *R. mucronata* seedlings grown under full sunlight (HL), 50% shade (ML) and 80% shade (LL) conditions. Values are means ± SD (n=3-4 plants). Means in the same month, followed by different letters indicated significant dIfferences between shade regimes ($P<0.05$; Tukey HSD's test)

Fig. 11 Comparison of Fv/Fm ratio for leaves of *R. mucronata* seedlings grown under full sunlight (HL), 50% shade (ML) and 80% shade (LL) conditions. Values are means ± SD (n=3-4 plants). Means in the same month, followed by different letters indicated significant differences between shade regimes ($P<0.05$; Tukey HSD's test)

Figure 1
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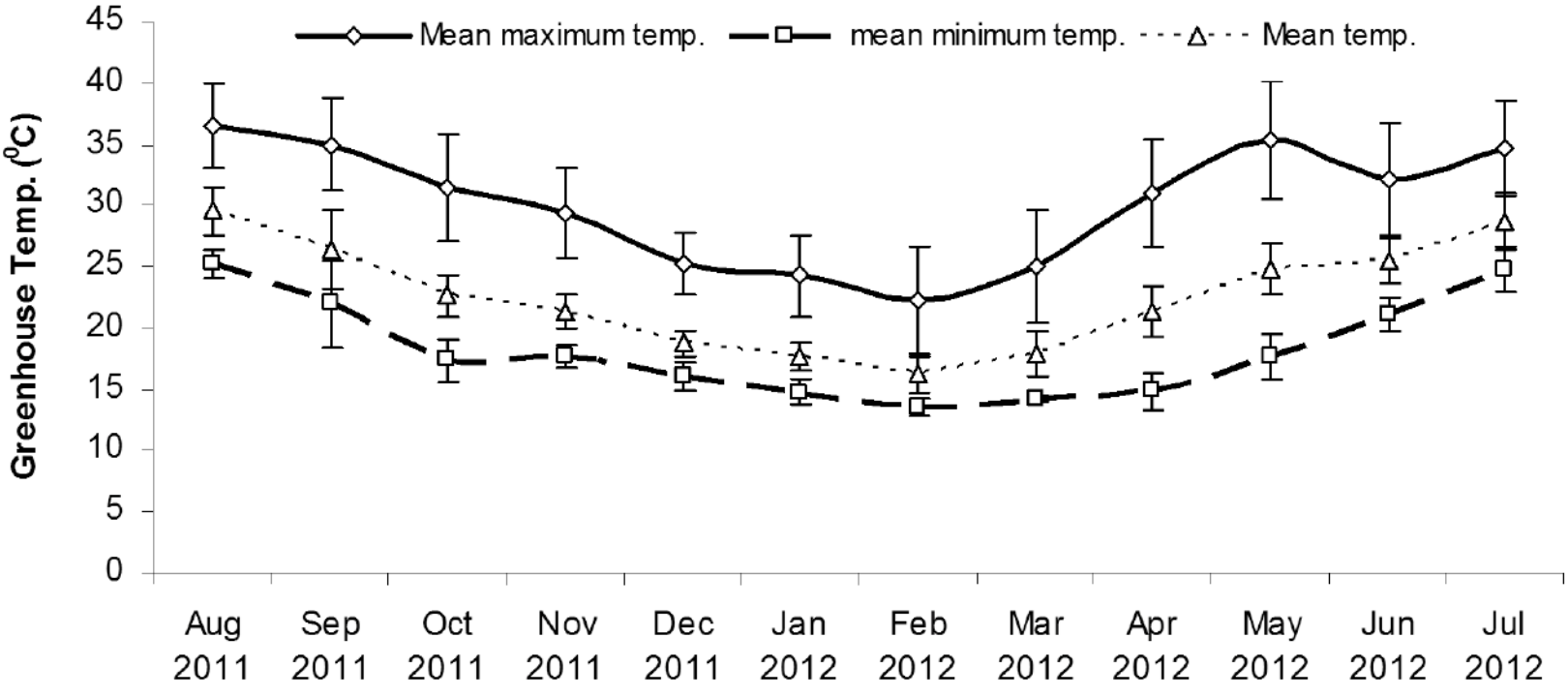

Figure 2
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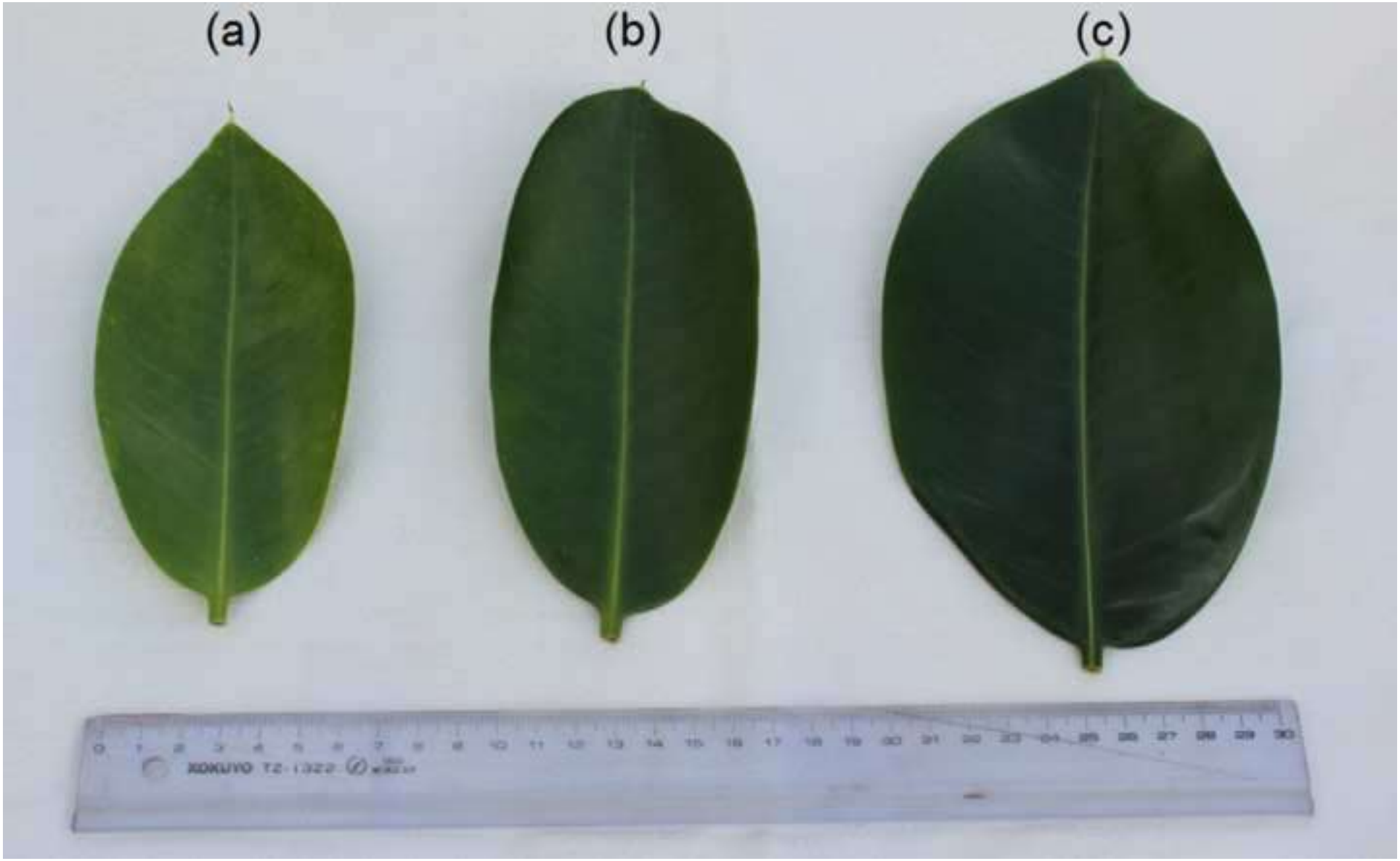

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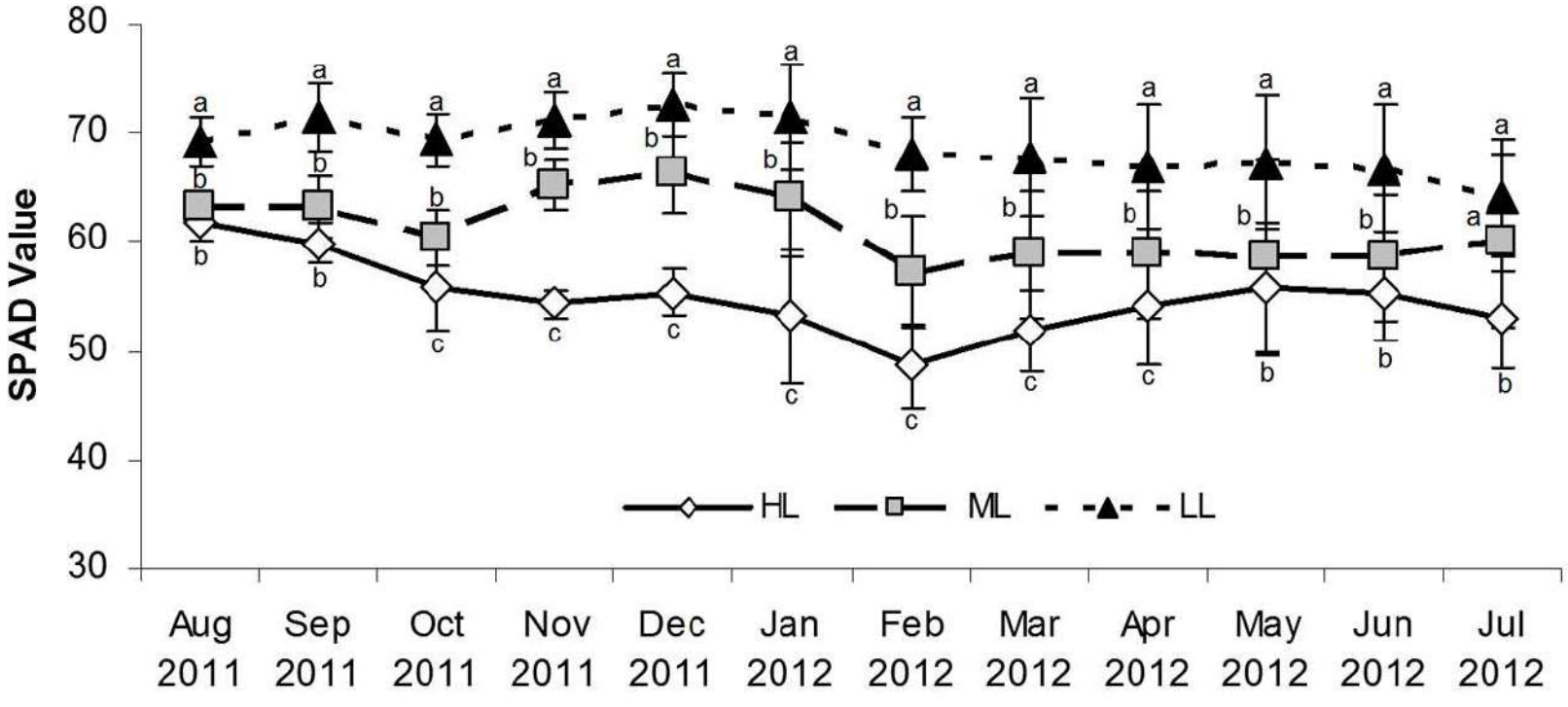

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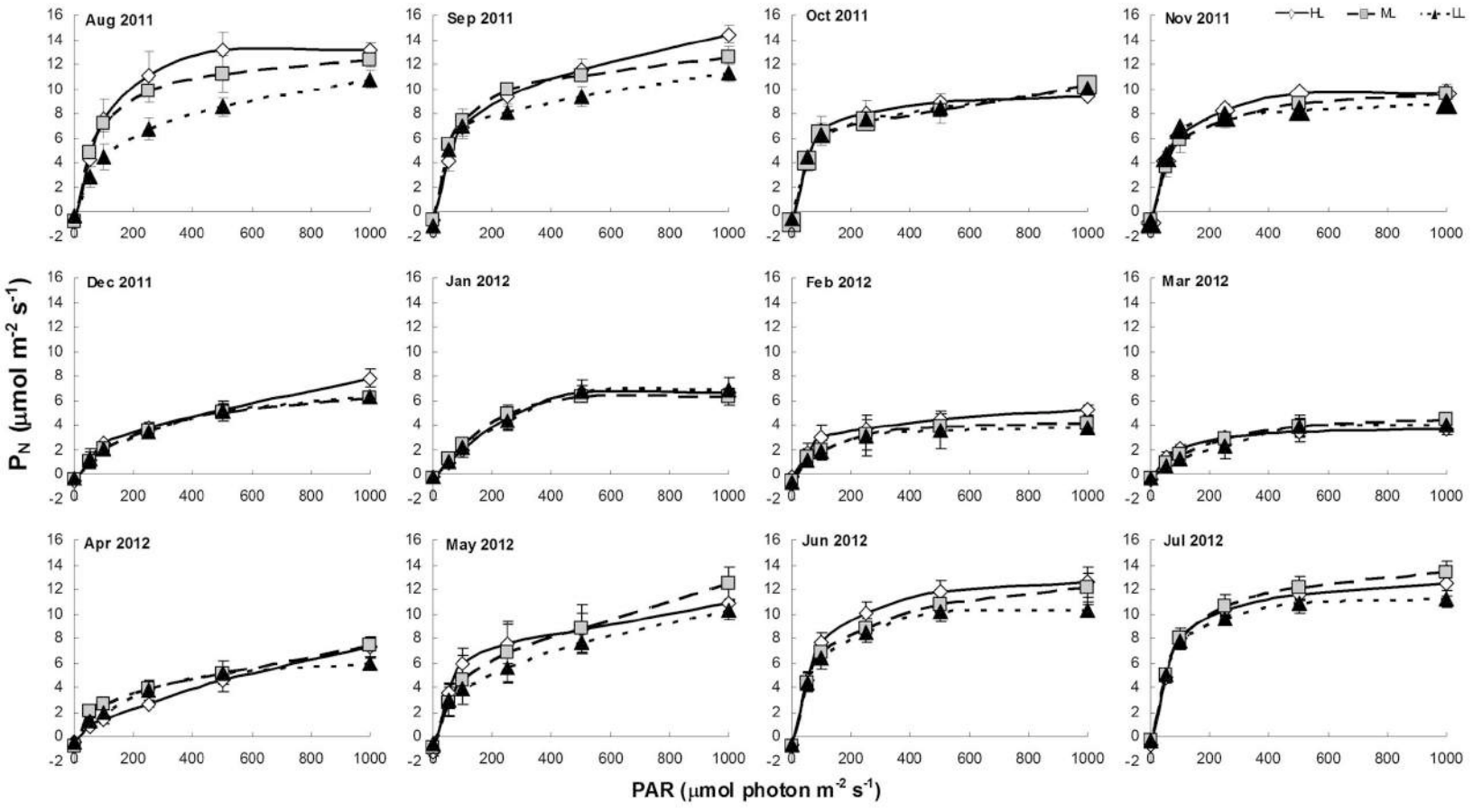

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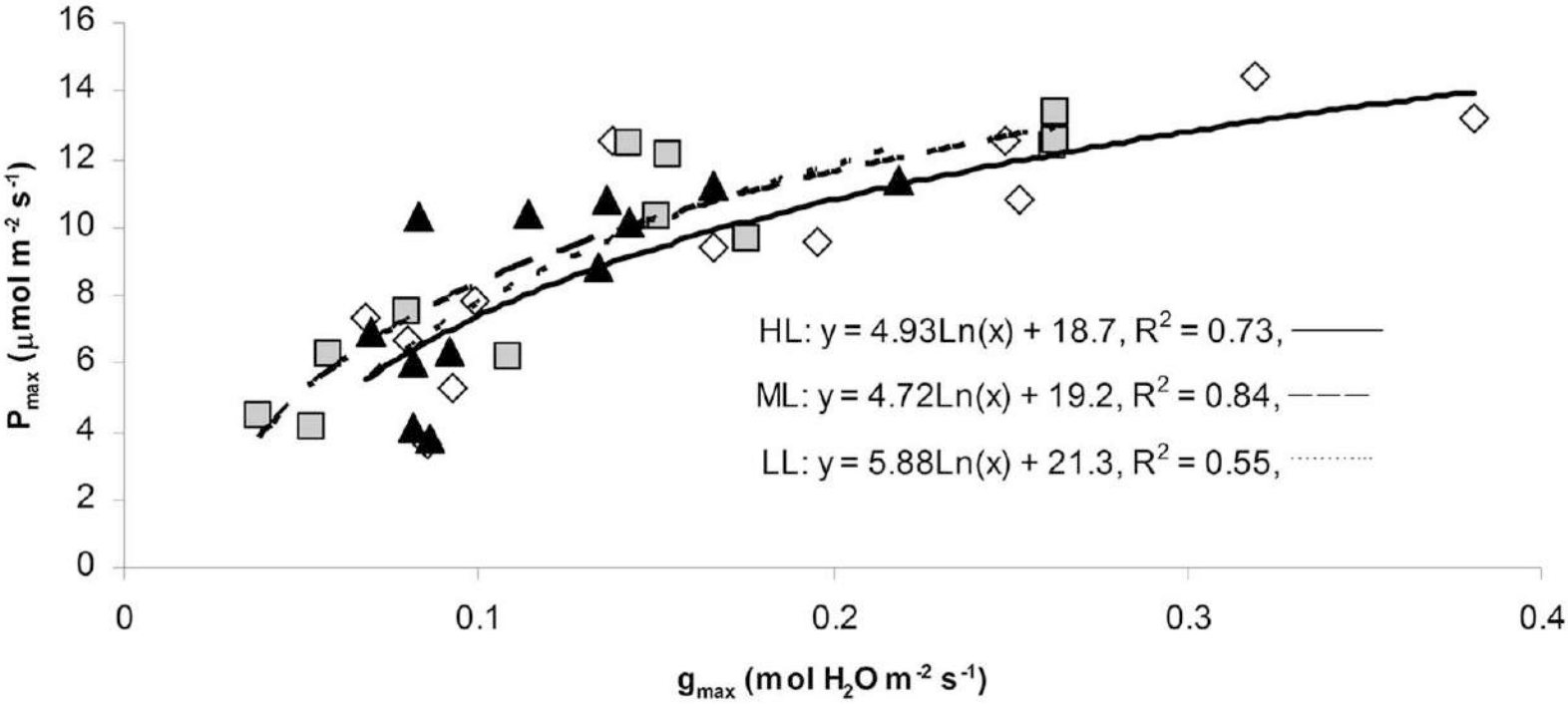

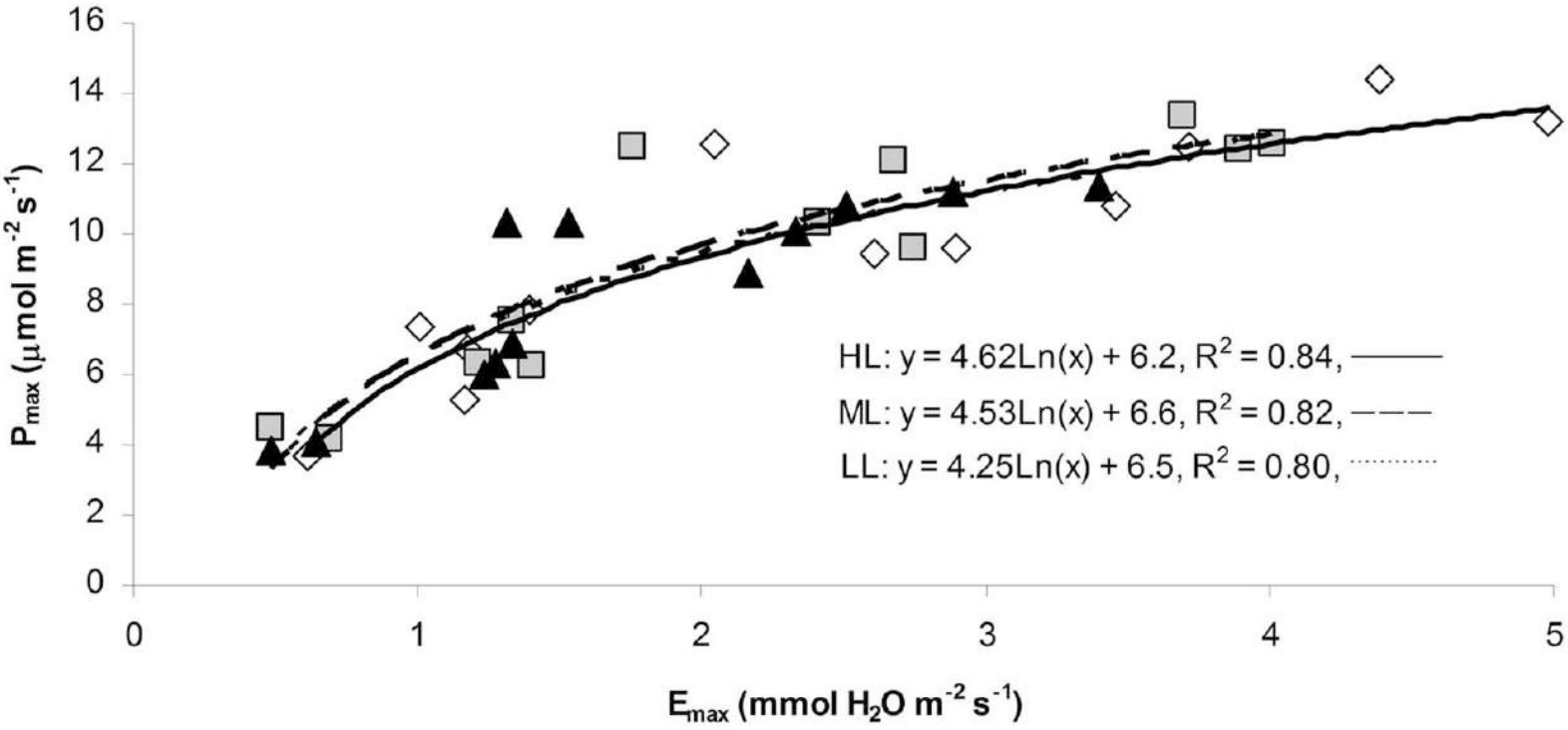

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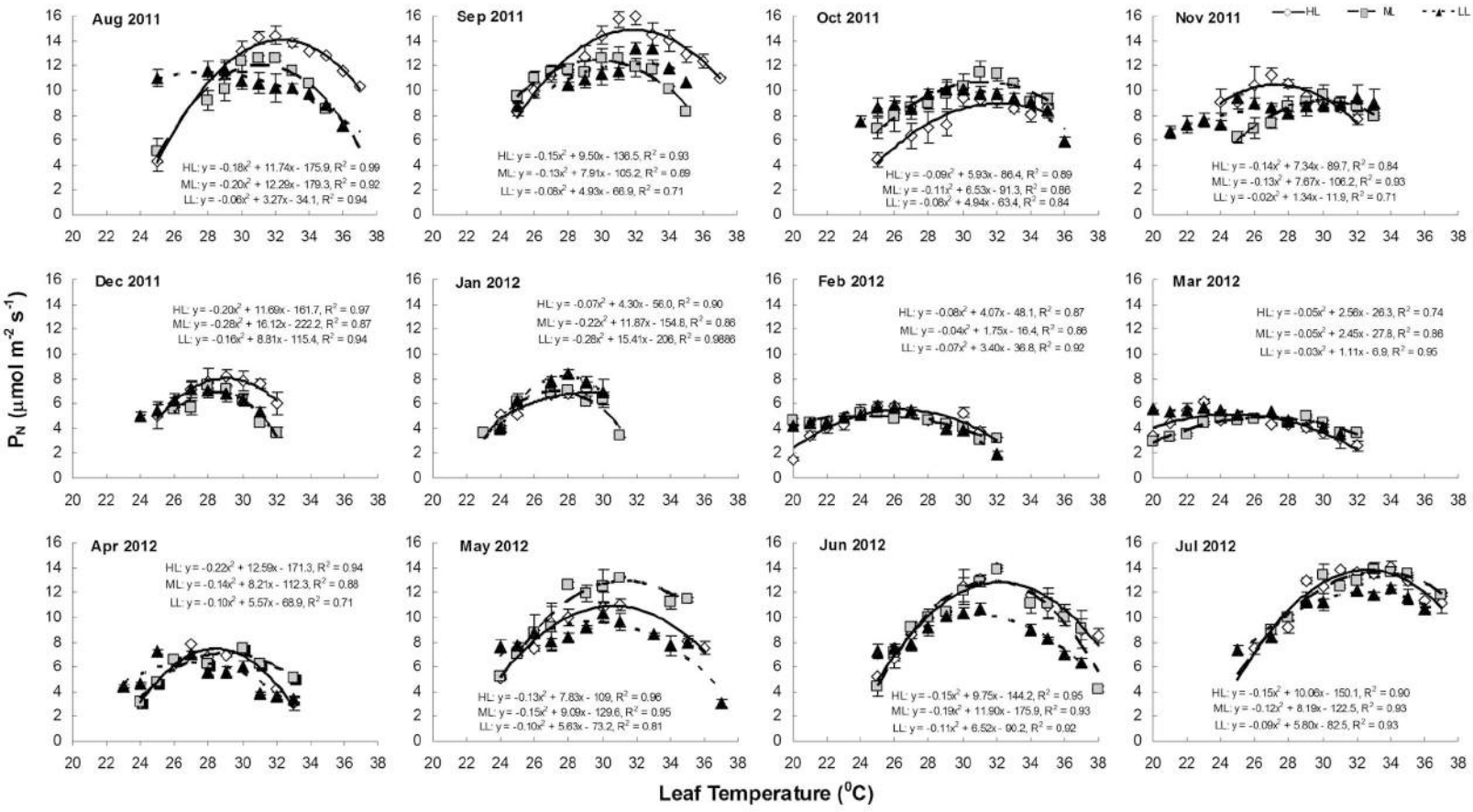

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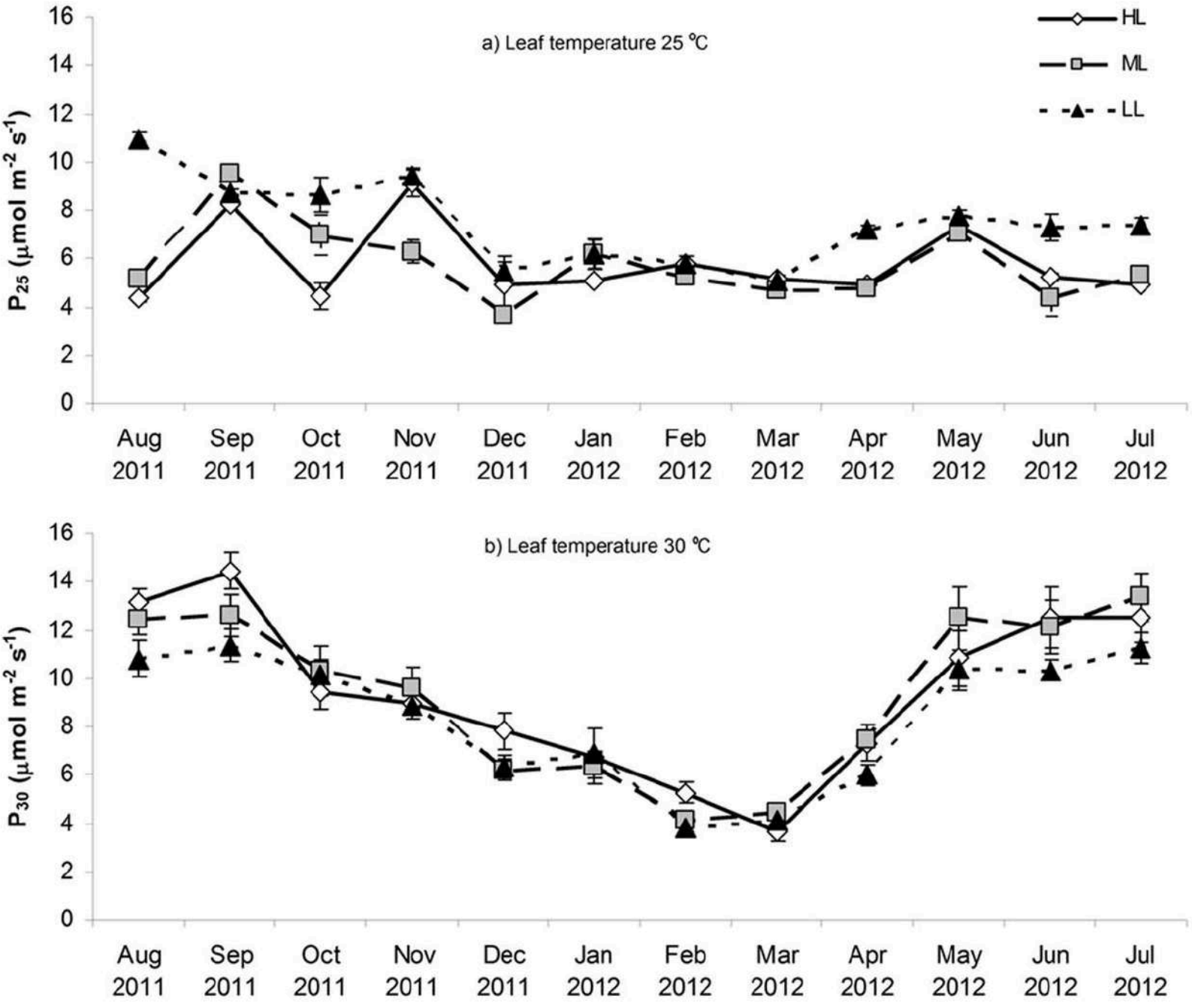

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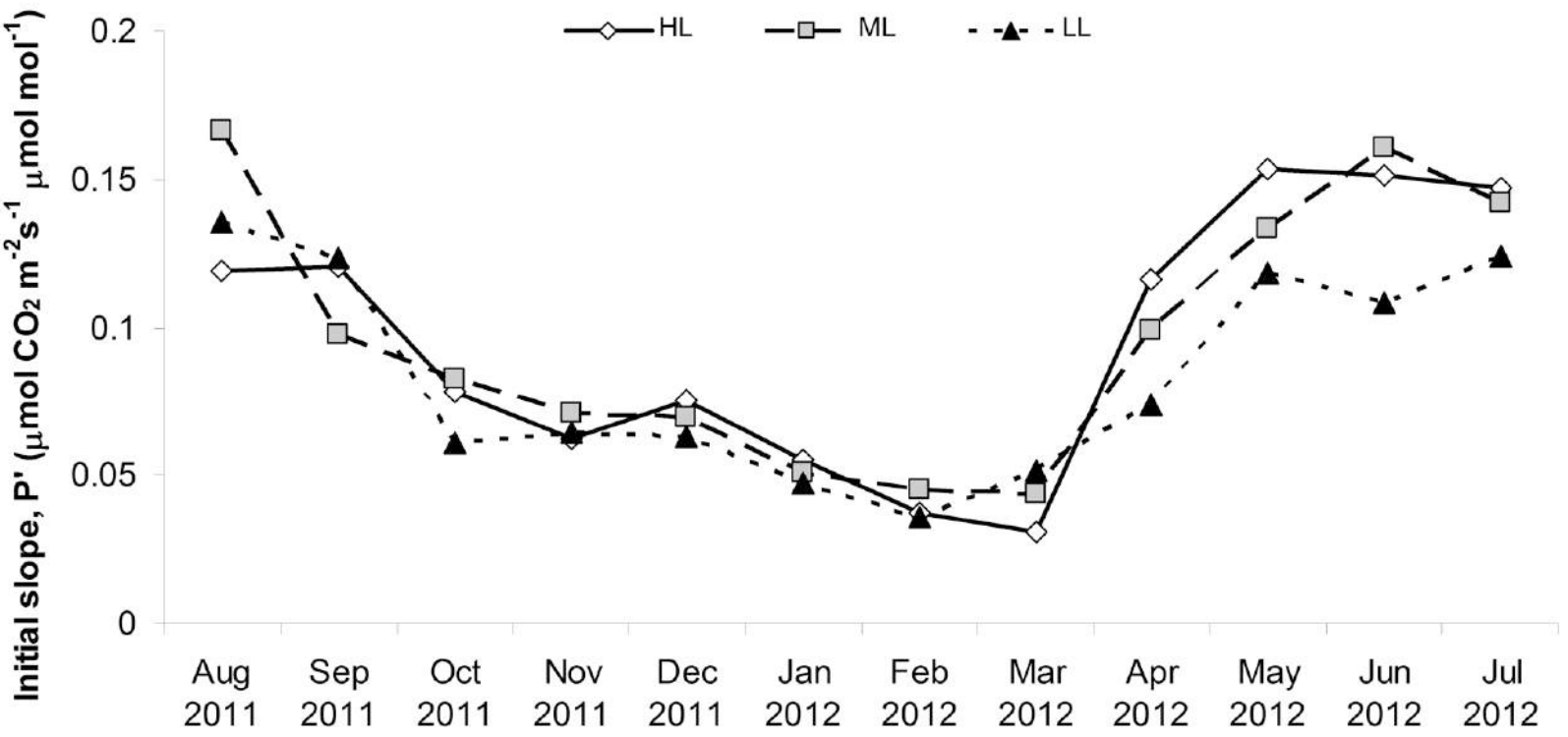

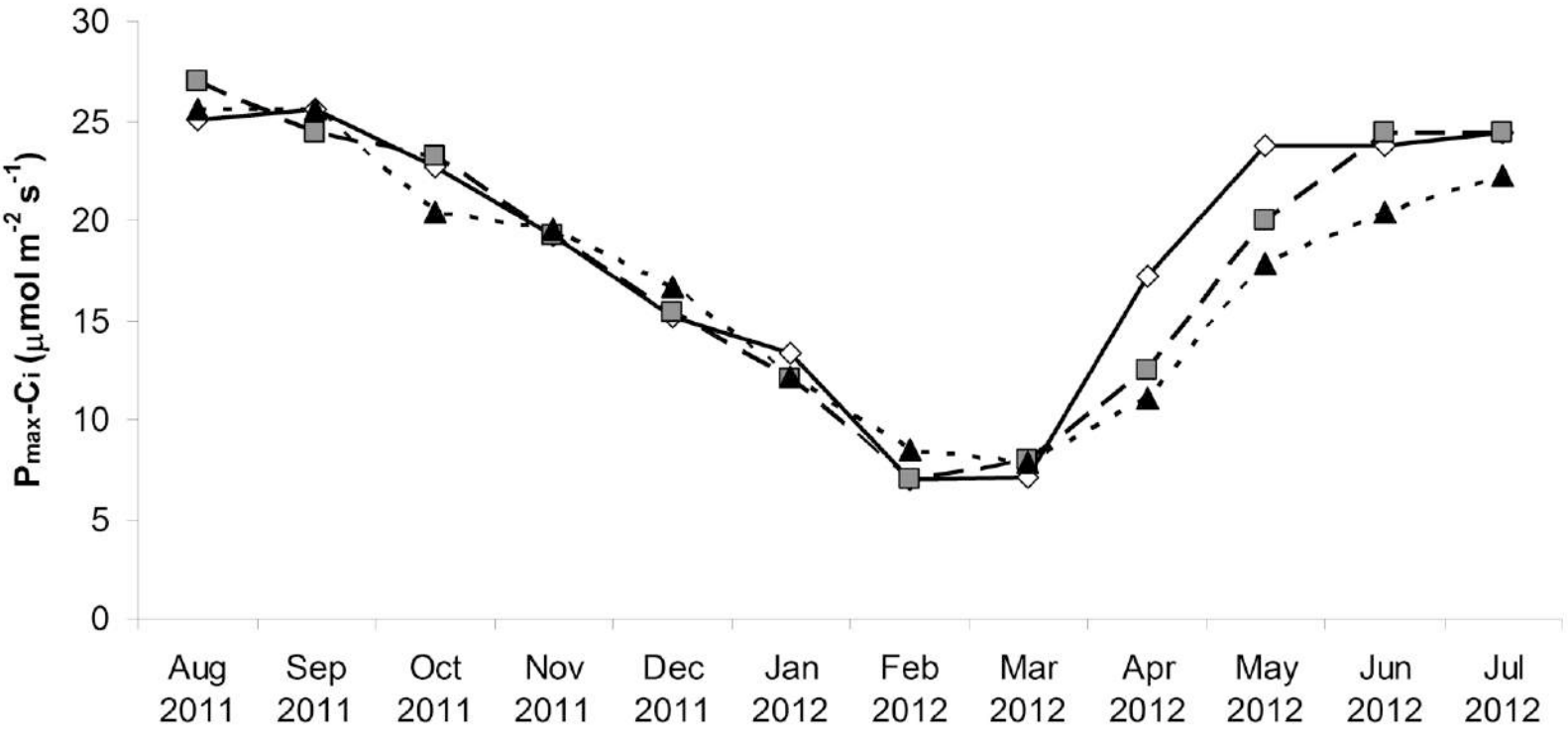

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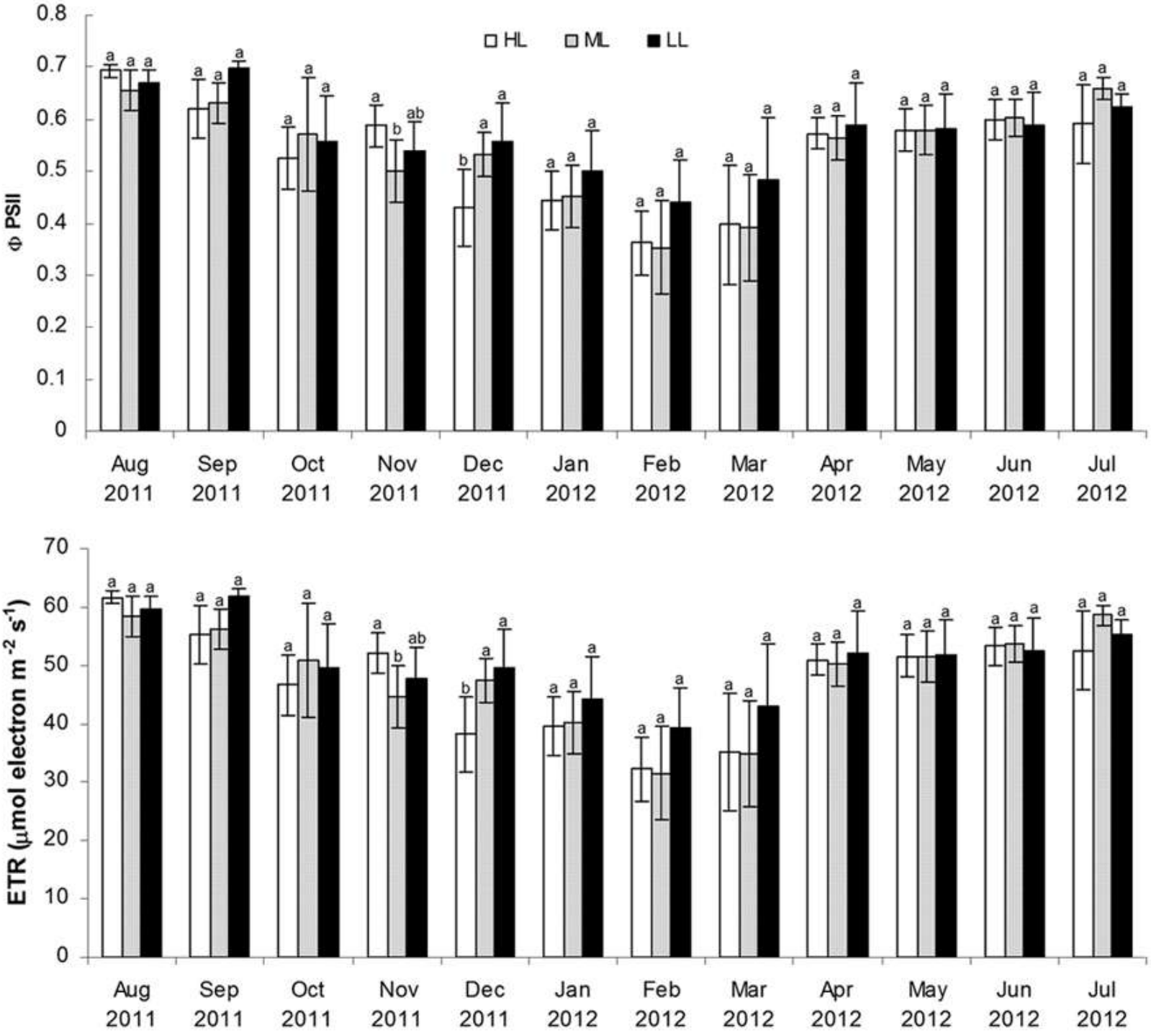

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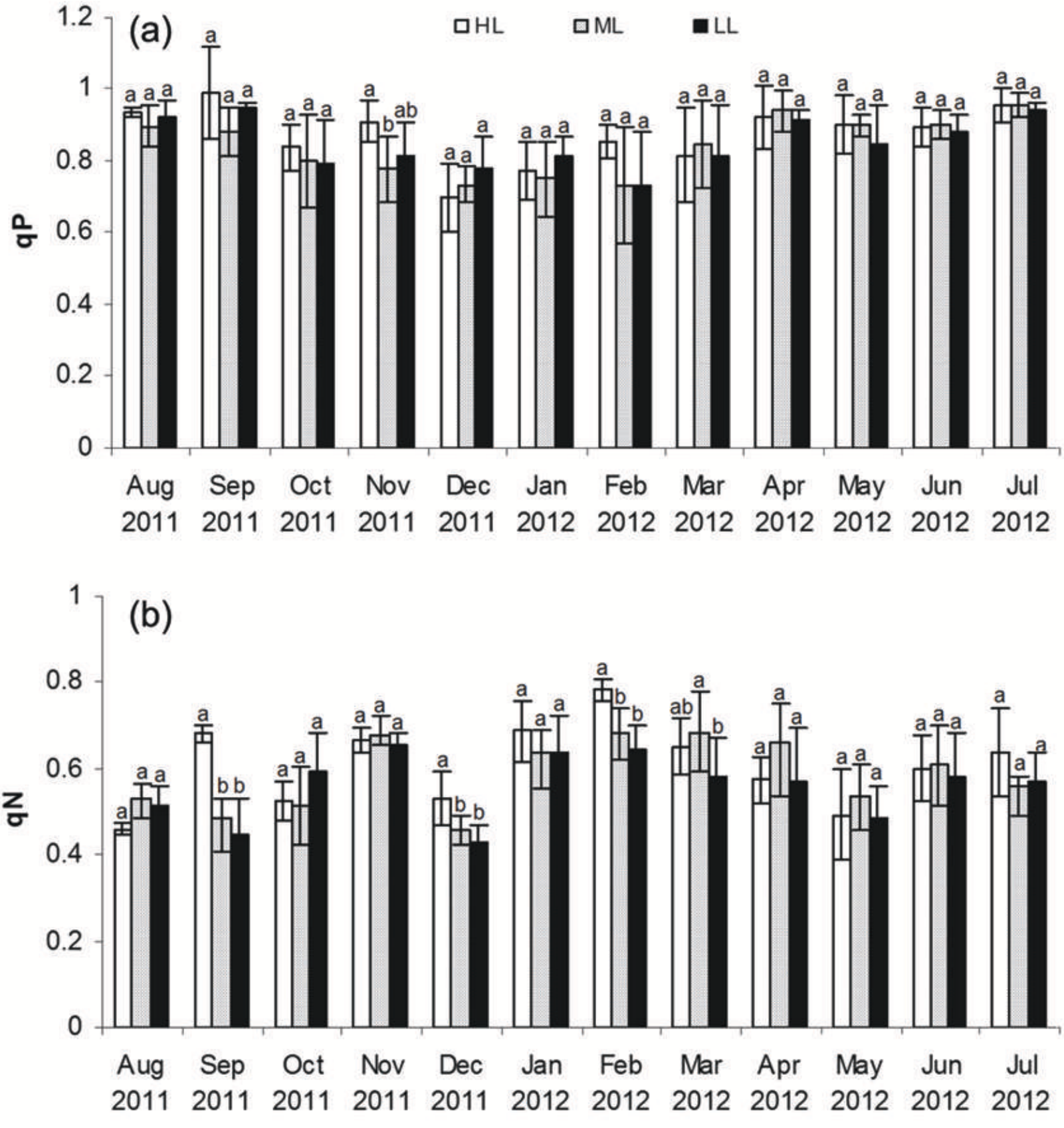

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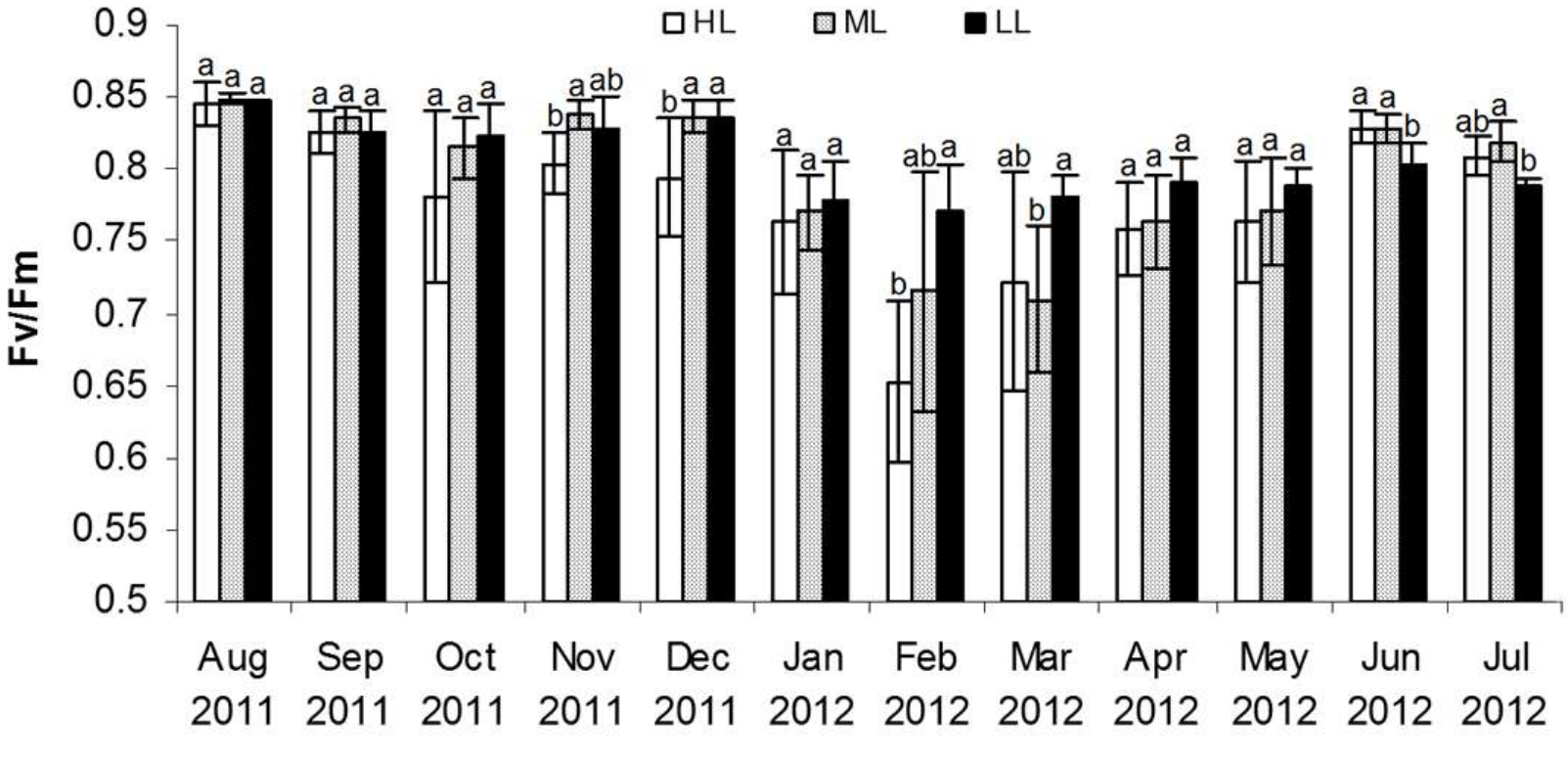

Table 1. The values of P_{max}, $g_{s\text{-}max}$, E_{max} and $C_{i\text{-}min}$ at saturating level of PAR 1000 μmol photons m^{-2} s^{-1} and leaf temperature 30 ^{0}C in leaves of *R. mucronata* grown under full sunlight (HL), 50% shade (ML), and 80% shade (LL) conditions. The functions were fitted to the points up to the maximum value for P_N, g_s and E at the saturation value based on Eq. 1.

Code	Month	Equation			P_{max}	$g_{s\text{-}max}$	E_{max}
		P_N	g_s	E			
HL	Aug 2011	P_N=I/(4.85+0.07I)	g_s=I/(82.08+2.44I)	E=I/(2.74+0.20I)	13.18	**0.40**	**4.93**
	Sep 2011	P_N=I/(12.23+0.06I)	g_s=I/(325.56+2.81I)	E=I/(15.95+0.21I)	**14.42**	0.32	4.43
	Oct 2011	P_N=I/(6.45+0.10I)	g_s=I/(126.62+5.89I)	E=I/(8.31+0.38I)	9.44	0.17	2.58
	Nov 2011	P_N=I/(7.45+0.09I)	g_s=I/(152.773+4.76I)	E=I/(7.89+0.34 I)	10.16	0.20	2.87
	Dec 2011	P_N=I/(43.95+0.08I)	g_s=I/(6213.07+3.88I)	E=I/(204.92+0.51I)	7.82	0.10	1.40
	Jan 2012	P_N=I/(20.50+0.13I)	g_s=I/(4123.21+8.36I)	E=I/(118.21+0.73I)	6.87	0.08	1.18
	Feb 2012	P_N=I/(26.30+0.16I)	g_s=I/(1764.07+9.04I)	E=I/(45.07+0.81I)	5.25	0.09	1.17
	Mar 2012	P_N=I/(23.51+0.24I)	g_s=I/(1742.51+9.56I)	E=I/(86.8+0.81I)	3.74	0.09	1.12
	Apr 2012	P_N=I/(81.19+0.06I)	g_s=I/(3260.60+11.34I)	E=I/(615.12+0.38I)	7.34	0.07	1.00
	May 2012	P_N=I/(9.72+0.083I)	g_s=I/(112.97+3.67I)	E=I/(12.49+0.28I)	10.83	0.26	3.42
	Jun 2012	P_N=I/(5.66+0.07I)	g_s=I/(11.00+6.05I)	E=I/(27.00+0.46I)	12.54	0.16	2.05
	Jul 2012	P_N=I/(5.85+0.07I)	g_s=I/(92.61+3.93I)	E=I/(16.43+0.0.25I)	12.49	0.25	3.75
ML	Aug 2011	P_N=I/(6.73+0.07I)	g_s=I/(129.04+3.28I)	E=I/(3.10+0.25I)	12.33	0.29	3.95
	Sep 2011	P_N=I/(6.73+0.07I)	g_s=I/(82.40+3.24I)	E=I/(4.13+0.24I)	12.33	**0.30**	**4.10**
	Oct 2011	P_N=I/(10.23+0.09I)	g_s=I/(55.86+5.78I)	E=I/(2.22+0.38I)	10.28	0.17	2.62
	Nov 2011	P_N=I/(9.78+0.09I)	g_s=I/(293.92+4.26I)	E=I/(16.41+0.28I)	9.64	0.22	3.37
	Dec 2011	P_N=I/(41.28+0.12I)	g_s=I/(819.29+8.41I)	E=I/(111.38+0.60I)	6.20	0.11	1.41
	Jan 2012	P_N=I/(14.93+0.13I)	g_s=I/(1934.98+11.57I)	E=I/(57+0.58I)	6.87	0.07	1.57
	Feb 2012	P_N=I/(22.82+0.22I)	g_s=I/(359.04+12.69I)	E=I/(81.37+1.37I)	4.13	0.08	0.69
	Mar 2012	P_N=I/(39.52+0.19I)	g_s=I/(3290.72+23.11I)	E=I/(55.79+1.01I)	4.45	0.04	0.94
	Apr 2012	P_N=I/(41.32+0.09I)	g_s=I/(1194.92+11.34I)	E=I/(78.20+0.67I)	7.48	0.08	1.34
	May 2012	P_N=I/(21.70+0.06I)	g_s=I/(287.65+6.72I)	E=I/(56.29+0.51I)	12.48	0.14	1.77
	Jun 2012	P_N=I/(10.18+0.07I)	g_s=I/(20.00+6.50I)	E=I/(40.54+0.33I)	12.10	0.15	2.70
	Jul 2012	P_N=I/(6.382+0.07I)	g_s=I/(114.04+3.69I)	E=I/(10.68+0.25I)	**13.37**	0.26	3.84
LL	Aug 2011	P_N=I/(18.45+0.07I)	gs=I/(870.52+6.26I)	E=I/(59.80+0.341I)	10.82	0.14	2.50
	Sep 2011	P_N=I/(11.54+0.08I)	gs=I/(13.00+4.60I)	E=I/(0.75+0.29I)	**11.35**	**0.22**	**3.44**
	Oct 2011	P_N=I/(5.19+0.10I)	g_s=I/(107.65+6.28I)	E=I/(0.6+0.43I)	9.88	0.16	2.32
	Nov 2011	P_N=I/(5.32+0.11I)	g_s=I/82.27+6.34I)	E=I/(9.55+0.37I)	8.82	0.16	2.63
	Dec 2011	P_N=I/(36.61+0.12I)	g_s=I/(1748.05+9.16I)	E=I/(175.2+0.61I)	6.34	0.09	1.27
	Jan 2012	P_N=I/(14.93+0.13I)	g_s=I/(1175.72+13.23I)	E=I/(140.17+0.60I)	6.87	0.07	1.35
	Feb 2012	P_N=I/(17.51+0.25I)	g_s=I/(1284.39+10.33I)	E=I/(157.69+1.08I)	3.80	0.09	0.81
	Mar 2012	P_N=I/(50.41+0.20I)	g_s=I/(728.15+9.52I)	E=I/(711.87+0.85I)	4.07	0.10	0.64
	Apr 2012	P_N=I/(32.26+0.13I)	g_s=I/(887.56+11.37I)	E=I/(111.15+0.70I)	6.01	0.08	1.23
	May 2012	P_N=I/(26.88+0.07I)	g_s=I/(395.25+8.37I)	E=I/(37.76+0.61I)	10.35	0.11	1.54
	Jun 2012	P_N=I/(6.78+0.09I)	g_s=I/(173.69+10.76I)	E=I/(245.45+0.51I)	10.33	0.09	1.32
	Jul 2012	P_N=I/(4.41+0.09I)	g_s=I/(192.88+4.98I)	E=I/(14.68+0.33I)	11.22	0.19	2.90

Author contribution

A. Nose designed and supervised the whole research work. TZ. Ulqodry and F. Matsumoto conducted the experiment, analyzed data and wrote the manuscript draft. Y. Okimoto and SH. Zheng corrected some parts of the manuscript.

Fw: ACPP: Your manuscript entitled Study on Photosynthetic Responses and Chlorophyll Fluorescence in Rhizophora mucronata seedlings under Shade Regime

Dari: 野瀬　昭博 (nosea@cc.saga-u.ac.jp)

Kepada: zia_uul@yahoo.com

Tanggal: Selasa, 28 Januari 2014 pukul 06.29 WIB

-----Original Message-----
From: Zoltan Gombos
Sent: Monday, January 27, 2014 10:21 PM
To: Akihiro Nose
Subject: ACPP: Your manuscript entitled Study on Photosynthetic Responses and Chlorophyll Fluorescence in Rhizophora mucronata seedlings under Shade Regimes

Ref.: Ms. No. ACPP-D-13-00947R1
Study on Photosynthetic Responses and Chlorophyll Fluorescence in Rhizophora mucronata seedlings under Shade Regimes
Acta Physiologiae Plantarum

Dear Dr Nose,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

The reviewers' comments can be found at the end of this email or can be accessed by following the provided link.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript.

Your revision is due by 27-04-2014.

To submit a revision, go to http://acpp.edmgr.com/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Please note that this letter is a recommendation only, and the final decision is the sole responsibility of the Editor-in-Chief.

Yours sincerely

Grzegorz Marszalkowski
Editorial Office
Acta Physiologiae Plantarum

Reviewers' comments:

Reviewer #1: The paper still needs revisions at several points:
The abstract should be rewritten, it is not understandable in its present form.
Results:
I already suggested in my first review that instead of averaging over months, which brings into the experiments an unnecessary fluctuation, and may decrease the otherwise existing variation, should not be used. The authors evidently made a very careful and detailed work, they have daily data, I do not understand, why they do not use them fully. (In the attached pdf file, there are detailed comments and suggestions, about this problem.)
At several points in the Results, there are claimed variations, which are not supported by the present figures. Please if you agree with those comments, introduce those into the Discussion also (Again, see my comments in the attached file).

I still had problems with the English usage at several points, and I made suggestions in the attached file. Since my mother language is not English

either, please accept them only if you agree fully with them, and if possible seek the advice of an English-speaking person.

Reviewer #2: The MS has been improved according to my suggestions. I accept the revised version.

I would suggest a basic correction according to Reviewer #1. It is still not acceptable at the present form.

There is additional documentation related to this decision letter. To access the file(s), please click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.
http://acpp.edmgr.com/l.asp?i=87940&l=SCGD7HJF

Fw: ACPP: Your manuscript entitled Study on Photosynthetic Responses and Chlorophyll Fluorescence in Rhizophora mucronata seedlings under Shade Regime

Dari: 野瀬　昭博 (nosea@cc.saga-u.ac.jp)

Kepada: zia_uul@yahoo.com

Tanggal: Rabu, 30 April 2014 pukul 15.32 WIB

-----Original Message-----
From: Przemyslaw Wojtaszek
Sent: Wednesday, April 30, 2014 5:52 AM
To: Akihiro Nose
Subject: ACPP: Your manuscript entitled Study on Photosynthetic Responses and Chlorophyll Fluorescence in Rhizophora mucronata seedlings under Shade Regimes

Ref.: Ms. No. ACPP-D-13-00947R2
Study on Photosynthetic Responses and Chlorophyll Fluorescence in Rhizophora mucronata seedlings under Shade Regimes
Acta Physiologiae Plantarum

Dear Dr Nose,

I am pleased to tell you that your work has now been accepted for publication in Acta Physiologiae Plantarum.

Thank you for submitting your work to this journal.

With kind regards

Przemyslaw Wojtaszek
Editor-in-Chief
Acta Physiologiae Plantarum

Comments:

Reviewer #1: The authors made the necessary changes, I suggest the acceptation of the manuscript.

Fw: Proofs for your article in ACTA PHYSIOLOGIAE PLANTARUM (1566)

Dari: 野瀬　昭博 (nosea@cc.saga-u.ac.jp)

Kepada: zia_uul@yahoo.com

Tanggal: Minggu, 18 Mei 2014 pukul 06.38 WIB

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