Tengku Zia Ulqodry <zia_uul@unsri.ac.id>

Decision on manuscript CMJS.25.08.18-9480

5 pesan

WASU PATHOM-AREE <wasu.p@cmu.ac.th> 23 Agustus 2019 pukul 16.02
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Cc: WASU PATHOM-AREE <wasu.p@cmu.ac.th>

Dear Authors,

Manuscript Number: **CMJS.25.08.18-9480**

Title: **Short-term impacts of soaking periods and NaCl concentrations to photosynthetic O_2 evolution and CO_2 uptake of mangrove seedlings from East Sumatera Coastline of Indonesia**

Thank you for submitting your paper to Chiang Mai Journal of Science. I have now received the reports from the referees who provided very constructive comments. The comments are included at the bottom of this letter. I am pleased to inform you that the referees have recommended publication as a **Research Article**, but also suggest **Major revisions** to your manuscript. Therefore, I invite you to respond to their comments point-by-point in the cover letter and revise your manuscript accordingly. In your revised manuscript please highlight any amendments and submit a list of changes that had been made.

Please submit the revised version of your manuscript to me within 4 weeks, preferably by 22 September 2019 otherwise the paper will be treated as a new submission. If you need longer time than this, please contact me to agree on an alternative deadline. If you not intend to submit a revised version of manuscript to Chiang Mai Journal of Science, you must withdraw this submission before sending the paper to another journal.

Yours sincerely,
Wasu Pathom-aree

Asst. Prof. Dr. Wasu Pathom-aree

Editor-in-Chief Chiang Mai Journal of Science

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CMJS.25.08.18-9480 from reviewer2.doc
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Tengku Zia Ulqodry <zia_uul@unsri.ac.id> 22 September 2019 pukul 09.39
Kepada: WASU PATHOM-AREE <wasu.p@cmu.ac.th>
Cc: zia uul <zia_uul@yahoo.com>

Dear Ass. Prof. Dr. Wasu Pathom-aree
Editor-in-Chief Chiang Mai Journal of Science

Thank you for the Decision letter and also the recommendation.

Due to this manuscript was recommended to be Research Article with Major Revisions, We need additional time 1 week (preferably by 29 September 2019) to discuss between authors in order to revise and improve the manuscript .

Thank You for your understanding.

Yours sincerely

Tengku Zia Ulqodry
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[Kutipan teks disembunyikan]

WASU PATHOM-AREE <wasu.p@cmu.ac.th> 23 September 2019 pukul 10.04
Kepada: Tengku Zia Ulqodry <zia_uul@unsri.ac.id>

Dear Dr. Ulqodry,

Many thanks for your e-mail. Your deadline is now extended to 30 September 2019.

Yours sincerely,
Wasu Pathom-aree

Asst. Prof. Dr. Wasu Pathom-aree

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Subject: Re: Decision on manuscript CMJS.25.08.18-9480

[Kutipan teks disembunyikan]

Tengku Zia Ulqodry <zia_uul@unsri.ac.id> 30 September 2019 pukul 21.42
Kepada: WASU PATHOM-AREE <wasu.p@cmu.ac.th>

Dear Ass. Prof. Dr. Wasu Pathom-aree
Editor-in-Chief of Chiang Mai Journal of Science and Reviewers

Thank you very much for reading our manuscript and giving us many kind advices. We would like to show our responses to editor and reviewers here, and provide the revised manuscript.

The blue and green parts in the revised manuscript were changed from the original one in order to respond the 1st and 2nd reviewers suggestion respectively.

Best regards

Tengku Zia Ulqodry
Corresponding Author
[Kutipan teks disembunyikan]

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2017K

WASU PATHOM-AREE <wasu.p@cmu.ac.th> 8 Oktober 2019 pukul 18.19
Kepada: Tengku Zia Ulqodry <zia_uul@unsri.ac.id>, WASU PATHOM-AREE <wasu.p@cmu.ac.th>

Dear Authors,

I am pleased to inform you that, following the satisfactory completion of your revisions in response to the comments of the referees, your paper **CMJS.25.08.18-9480 "Short-term impacts of soaking periods and NaCl concentrations to photosynthetic O_2 evolution and CO_2 uptake of mangrove seedlings from East Sumatera Coastline of Indonesia"** has now been accepted for publication as a **Research Article**. The article will be published in volume 47(1) due in January 2020. Reprints of your paper will be available for download free of charge from our journal website http://epg.science.cmu.ac.th/ejournal/.

On behalf of our Editorial Board, may I thank you very much indeed for publishing your work in our **Chiang Mai Journal of Science** and I sincerely hope that you will consider submitting further articles in the future.

Yours sincerely,
Wasu Pathom-aree

Asst. Prof. Dr. Wasu Pathom-aree

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Short-term impacts of soaking periods and NaCl concentrations to photosynthetic O_2 evolution and CO_2 uptake of mangrove seedlings from East Sumatera Coastline of Indonesia

Abstract. We clarified the photosynthetic performance of different mangrove zonation species (*Avicennia marina*, *Rhizophora mucronata*, and *Bruguiera gymnorrhiza*) under a combination of salinity and soaking stress by using a liquid-phase O_2 electrode combined with CO_2 optodes simultaneously. Photosynthesis O_2 evolution and CO_2 uptake for each mangrove seedlings showed different responses with increasing the soaking period and NaCl concentration. Among three mangrove species, photosynthetic performance in *B. gymnorrhiza* was decreased significantly as compared with other species. In other side, photosynthetic performance of *A. marina* was uniquely increased with increasing the soaking period and NaCl concentration. It showed that *A. marina* maintained the high photosynthetic rate even under the soaking condition. *R. mucronata* had an intermediate response to NaCl concentration during the soaking periods.

Keywords: CO_2 uptake, O_2 evolution, mangrove, photosynthetic rate, salinity, soaking tolerance.

Introduction

Mangrove is a major and unique coastal ecosystem in tropic area. They have a higher carbon fixation capacity than terrestrial forests [1], adaptation ability under abiotic stress [2], and specific habitat zonation [3]. Mangroves, which thrive luxuriantly in tidal saline wetlands, are especially adapted to salinity and submerged stresses [4].

Belong to the C_3 plant, mangroves also can be classified as "seaweed", since it can grow in high salinity and submerge conditions, whereas C_3 plants could not survive [5]. Furthermore, we use the term "soaking condition" to reflect the complete submerged condition whereas (where???) the leaves usually immersed in water column [6]. During soaking condition, the chances of plant to fix carbon and capture oxygen are restricted. This situation is worsened ~~due to~~ because the irradiance available to sustain underwater photosynthesis for survival is drastically reduced [7].

In recent decades, many workers have been interested in understanding how stress limits mangrove photosynthesis [4] [8] [9]. However, there are relatively few studies on the combined effects of salinity and soaking conditions ~~in~~ on mangrove photosynthetic performance [10].

Species differences in mangrove responses to the interactive effects of some stress conditions might explain important differences in mangrove forest structure [11]. *Avicennia marina, Rhizophora mucronata* and *Bruguiera gymnorrhiza* are three dominant mangrove species in East Sumatera coastlines, Indonesia. Based upon illations made from mangrove zonation, these three mangrove species appear to differ in their sensitivity to salinity and soaking on ion concentration, tissue water potential and chloroplast (author should re-write this part???) [12]. However, the photosynthetic performance between these three mangrove species ~~in responses to~~ in response to salinity and soaking conditions have not been well ~~known~~ studied. The mangrove photosynthetic responses to combined soaking-salinity effects could be useful to clarify the mangrove zonation pattern [13].

The estimation of mangrove photosynthetic gas exchange has been evaluated either by O_2 evolution or CO_2 uptake [14] but was limited under soaking conditions as the Infra-Red Gas Analyser is sensitive to water immersion [15]. The simple and stable measurement of mangrove leaf O_2 evolution and CO_2 uptake simultaneously under aqueous conditions have been held using the leaf-disc O_2 electrode with CO_2 optodes sensor [16].

The objective of this research was to investigate the impacts of soaking periods and NaCl concentrations ~~to~~ on photosynthetic O_2 evolution and CO_2 uptake of three mangrove species, i.e., *A. marina*, *R. mucronata* and *B. gymnorrhiza*. The photosynthetic responses from each mangrove species will be compared with their specific zonation.

Materials and Methods

Plant Materials

Mangrove propagules were collected from East Sumatera Coastline of Indonesia, *A. marina* propagules were collected from Banyuasin Peninsula (02^{o} 11' S, 104^{o} 53' E) while *B. gymnorrhiza* and *R. mucronata propagules* from Galang Island (0° 45' N, 104° 15' E). The propagules were initially grown in the greenhouse. After 5 months, seedlings with fully developed healthy leaves were subjected to treatments. Four levels of soaking periods treatment (15, 30, 60, and 120 min) were employed in each of the three levels of NaCl concentration treatment (100, 300, and 500 mM). There were no NaCl added and no soaking for control leaves. (table below should be deleted)

~~Soaking Periods (Min)~~	~~NaCl Concentration (salinity level)~~		
	~~100 mM (low)~~	~~300 mM (mid)~~	~~500 mM (high)~~
~~15~~	~~√~~	~~√~~	~~√~~
~~30~~	~~√~~	~~√~~	~~√~~
~~60~~	~~√~~	~~√~~	~~√~~
~~120~~	~~√~~	~~√~~	~~√~~

After the soaking and NaCl treatments, the leaf sample was sliced using a safety razor under 50 mM HEPES buffer containing 0.5 mM $CaSO_4$ [17], and transferred into the electrode chamber.

Photosynthetic O_2 evolution and CO_2 uptake

Photosynthetic O_2 evolution and CO_2 uptake of mangrove leaves were measured simultaneously as described in [15]. The measurement was held in a closed chamber using a Clark oxygen electrode polarographic sensor (Hansatech, UK) with a sensor of 'pCO2 mini' optodes system (PreSens GmbH, Germany). All measurements were carried out with 20 mM $NaHCO_3$ as carbon dioxide source at 25°C. A slide projector lamp with a calibrated quantum sensor (Hansatech, UK) was used as photosynthetically active radiation (PAR) source.

Photosynthetic response of mangrove leaves at various PAR levels was maintained in decreasing order from 1000 to 50 μmol m^{-2} s^{-1} by placing a projector lamp at various distance from the chamber. For a dark respiration measurement, the electrode chamber was wrapped in two layers of aluminium foil. The photosynthetic O_2 evolution and CO_2 uptake were calculated from the initial slopes of the curves during the periods of apparent linear photosynthetic activity**.** The maximum photosynthetic rate (P_{max}) ~~as the response of photosynthetic rate to light intensity~~ was calculated by using the rectangular hyperbola model [18] [9]. (check fonts here…)

What is the reason/advantage for measuring both CO2 fixation and O2 evolution? If stressful conditions had more negative effects on O2 evolution than on CO2 fixation (such as Figure 3), how do you interpret this?

Results

The light saturation points of all treatments were commonly at PAR level around 500-1000 μmol photon m^{-2} s^{-1} (Figures 1–3). Under control condition, the maximum photosynthetic oxygen evolution of *A. marina* was lower (11.05 μmol m^{-2} s^{-1}) than *B. gymnorrhiza* (11.92 μmol m^{-2} s^{-1}) and *R. mucronata* (13.10 μmol m^{-2} s^{-1}). However, the maximum photosynthetic rate responses indicated different responses while subjected to variation of soaking periods and NaCl concentrations (Figure 4).

During low (NaCl 100 mM) and mid salinity (NaCl 300 mM) under soaked condition, photosynthetic-light responses of *A. marina* did not differ significantly from the control. ~~had no significance rate with control~~. The photosynthetic O_2 evolution and CO_2 uptake in *A. marina* was uniquely increased with increasing the soaking period and NaCl concentration. It means the photosynthetic performance of *A. marina* was better under high salinity rather than control during soaking periods (Figure 1 and 4).

For *R. mucronata*, the soaking periods under low and mid salinity did not affect photosynthetic performance and maximum photosynthetic O_2 evolution and CO_2 uptake significantly (Figure 2 and 4). This result suggested that *R. mucronata*, like *A. marina*, was well adapted to soaking condition under moderate salinity. Under high salinity, photosynthesis of *R. mucronata* declined rapidly and the maximum photosynthetic O_2 evolution dropped more clearly than CO_2 uptake. (please discuss this observation in the DISCUSSION)

In contrast with *A. marina* and *R. mucronata*, all types of soaking periods affected on decreasing of photosynthetic CO_2 uptake and O_2 evolution in *B. gymnorrhiza* (Figure 3 and 4). Maximum photosynthetic rate of *B. gymnorrhiza* decreased simultaneously during soaking periods along salinity escalation. The lessening of maximum photosynthetic O_2 evolution was higher than maximum photosynthetic CO_2 uptake. (These two sentences should be rephrased) Photosynthetic performance of *B. gymnorrhiza* was lowest compared with other species while exposed to high PAR 1000 μmol photon m^{-2} s^{-1} under high salinity.

Discussion

Understanding potential photosynthetic performances of mangroves to soaking, salinity, and light were substantial role??? for diagnosing successful mangrove lives. This information hopefully will act as additional important elucidation of mangrove zonation pattern.

The photosynthetic rate-light performance of each mangroves species under control condition reflected that *A. marina* < *B. gymnorrhiza* < *R. mucronata* (Figures 1-3). This sentence should be re-written? Do you mean under control condition (no NaCl, no soaking) photosynthetic performance in response to light intensity was highest in *R. mucronata*, followed by *B. gymnorrhiza* and *A. marina*????? Clough [19] also found that the net photosynthesis performance to light flux density of *B. gymnorrhiza* was lower than *Rhizophora spp.* However, according to Kawamitsu et al. [20] the leaf photosynthetic rate of *A. marina* was higher than *B. gymnorrhiza*. The result here indicated that the photosynthetic pattern of these three mangroves varied while subjected to soaking conditions and salinity escalation.

It was substantial to note that low growth and photosynthetic rate was consequency of mangrove light relationship???? [11] [9], especially while interacted with other stressor like salinity [21] and soaking. Generally flooding stressed mangrove leaf seedlings than other organs [22]. In contrast, we found that all combinations of soaking and salinity did not depress the leaf photosynthetic rate-light response of *A. marina* seedlings ~~than other mangrove species~~ (Figure 1). This result was in agreement with Kawamitsu et al. finding [5], which obtained that the photosynthesis performance of *A. marina* was not depressed even when seedling plants were submerged everyday??. *A. marina* root system could filter seawater, allowing only fresh water to translocate to the above-ground plant parts, hence preserving the leaf photosynthetic apparatus [5].

Similar with *A. marina*, there was no significance effects of soaking conditions on reducing photosynthetic rate at low to middle salinity levels in *R. mucronata* (Figure 2). During low to mid soaking and salinity, the primary productivity of mangrove *Rhizophora* was not changed significantly [10] and seedlings growth was well-maintained [23]. *Rhizophora* maintained photosynthetic–light response in the moderate inundation and salinity through high stomatal conductance mechanism [23].

In contrast with *A. marina* and *R. mucronata*, photosynthetic performance of *B. gymnorrhiza* for all salinity levels was higher in non-soaking than soaking conditions (Figure 3). It suggested that soaking was stressful to *B. gymnorrhiza* seedlings. A low growth rate in flooded plants could be caused by the negative effects of flooding on photosynthesis ~~from the leaf to the plant level~~ [6]. Maximum photosynthetic O_2 evolution and CO_2 uptake of *B. gymnorhiza* under saline soaking periods were usually lower than control (non saline soaking periods) (Figure 4*a*). This result indicated that *B. gymnorhiza* was more intolerant to soaking saline condition than *A. marina* and *R. mucronata*. Seedlings of *B. gymnorrhiza* had a relatively low tolerance to soaking [24] and also risked facing oxidative damage due to waterlogging [25].

Rhizophora in natural habitat was more adaptive to soaking and salinity than *Bruguiera* [12]. Our result also supported that *R. mucronata* had the high maximum photosynthetic performance for both CO_2 uptake and photosynthetic O_2 evolution (Figure 4*b*). *Rhizopora* maintained high photosynthetic rate even under stress condition due to their water use efficiency might increase uniquely with decreasing leaf water potential [26]). (This sentence should be re-written, and more clearly stated about the relationship between photosynthesis and water status???) *R. mucronata*, ‘‘the intermediate gap phase mangrove species’’ had a role as main (dominant???) plant in tropical coastal area and produced high leaf litter [9].

Regarding on the maximum photosynthetic rate, we supposed that species differences in mangrove responses to soaking and salinity condition showed distinctions characteristic. Maximum photosynthetic CO_2 uptake and O_2 evolution of *A. marina* were enhanced under higher salinity and increasing soaking periods (Figure 4*c*). This study indicated that among the three species, *A. marina* is best adapted to tolerate all salinity levels and soaking conditions. *A. marina* as pioneer vegetation in mangrove ecosystem adapted to broader habitats than *B. gymnorrhiza* and *R. mucronata*. *A. marina* ability to accumulate and excrete salts might contribute to protecting its photosynthetic performance. This result was also in line with the report of Naidoo [12] that *Avicennia* maintained low stomatal resistance values and tissue water potentials, and high relative water content in order to adapt well to soaking and saline stress condition.

One potential cause of mangrove zonation is the differential ability of propagules to establish at different soaking condition [27]. Our study suggested that the photosynthetic CO2 uptake and O2 evolution of *B. Gymnorrhiza* < *R. mucronata* < *A. marina* by escalation of soaking periods and salinity level seem to be appropriate with mangrove natural zonation in Indonesia. (should rewrite this part to express more clearly the relationship between variation in photosynthesis performance of different species in relation to zonation, growth and impact on mangrove forest structure)

Acknowledgments

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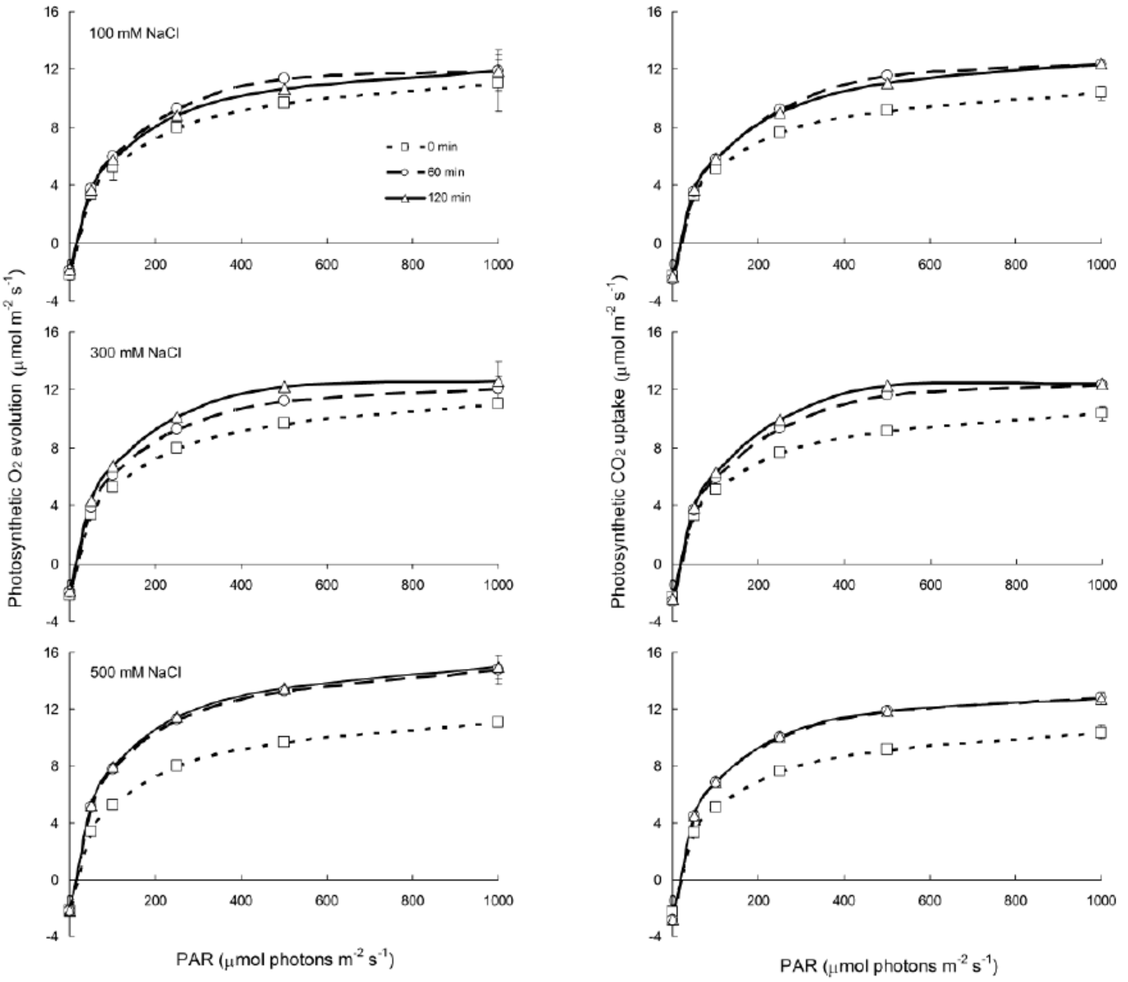

Figure 1 Light response curves for photosynthetic O_2 evolution and CO_2 uptake of mangrove leaves *A. marina* subjected to variation of soaking periods and NaCl concentrations. Control leaves were not soaked (0 min) and no NaCl added. Values are means ± SD (n=3-4 plants).

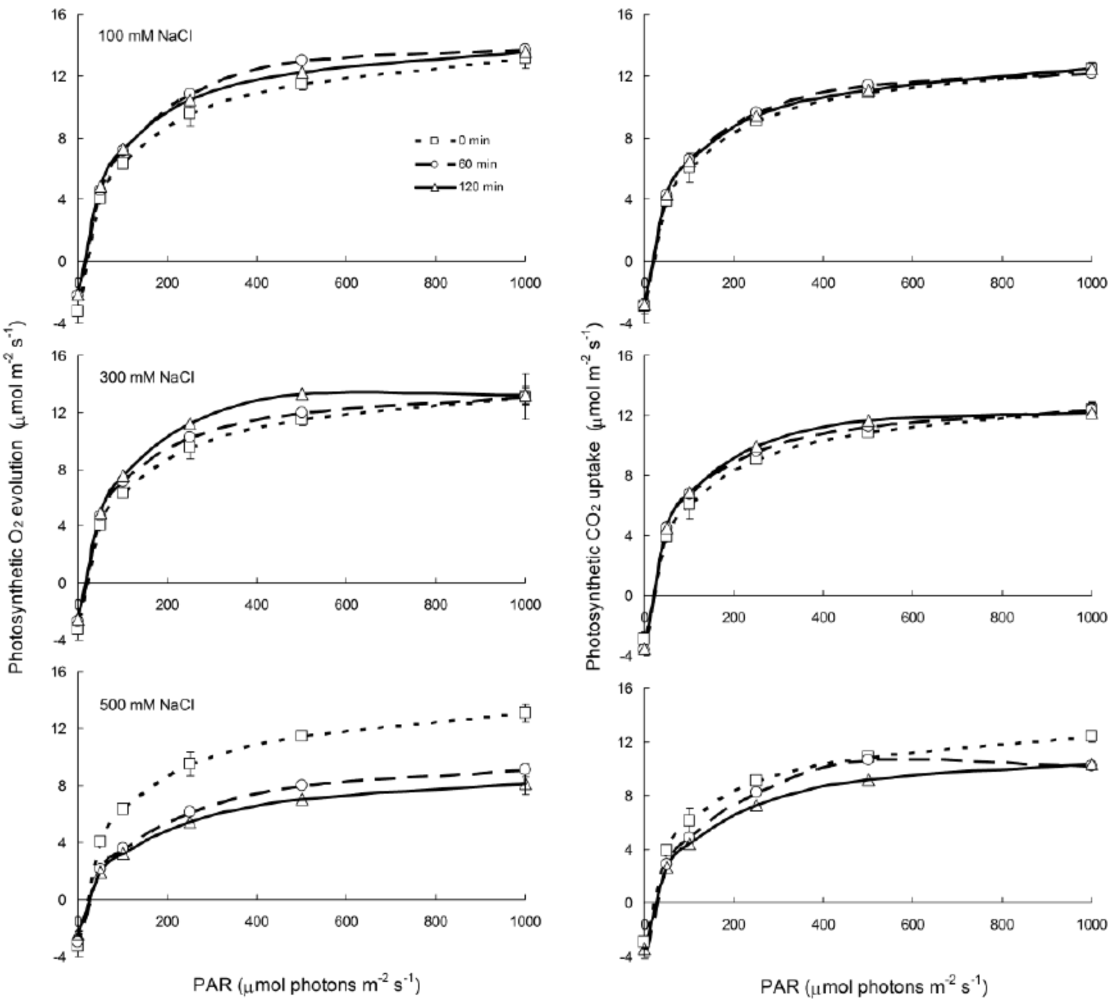

Figure 2 Light response curves for photosynthetic O_2 evolution and CO_2 uptake of mangrove leaves *R. mucronata* subjected to variation of soaking periods and NaCl concentrations. Control leaves were not soaked (0 min) and no NaCl added. Values are means <u>+</u> SD (n=3-4 plants).

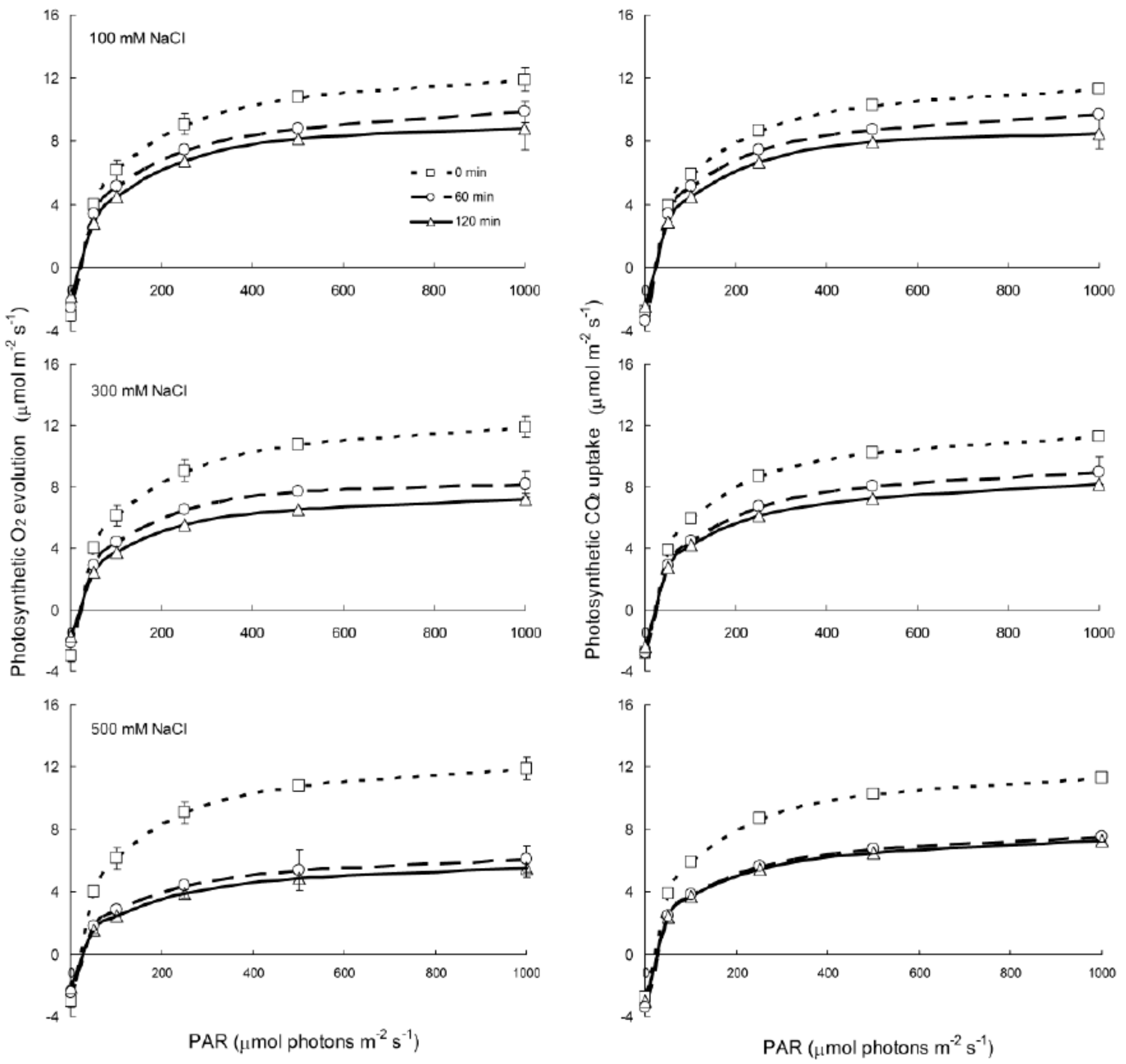

Figure 3 Light response curves for photosynthetic O_2 evolution and CO_2 uptake of mangrove leaves *B. gymnorrhiza* subjected to variation of soaking periods and NaCl concentrations. Control leaves were not soaked (0 min) and no NaCl added. Values are means ± SD (n=3-4 plants).

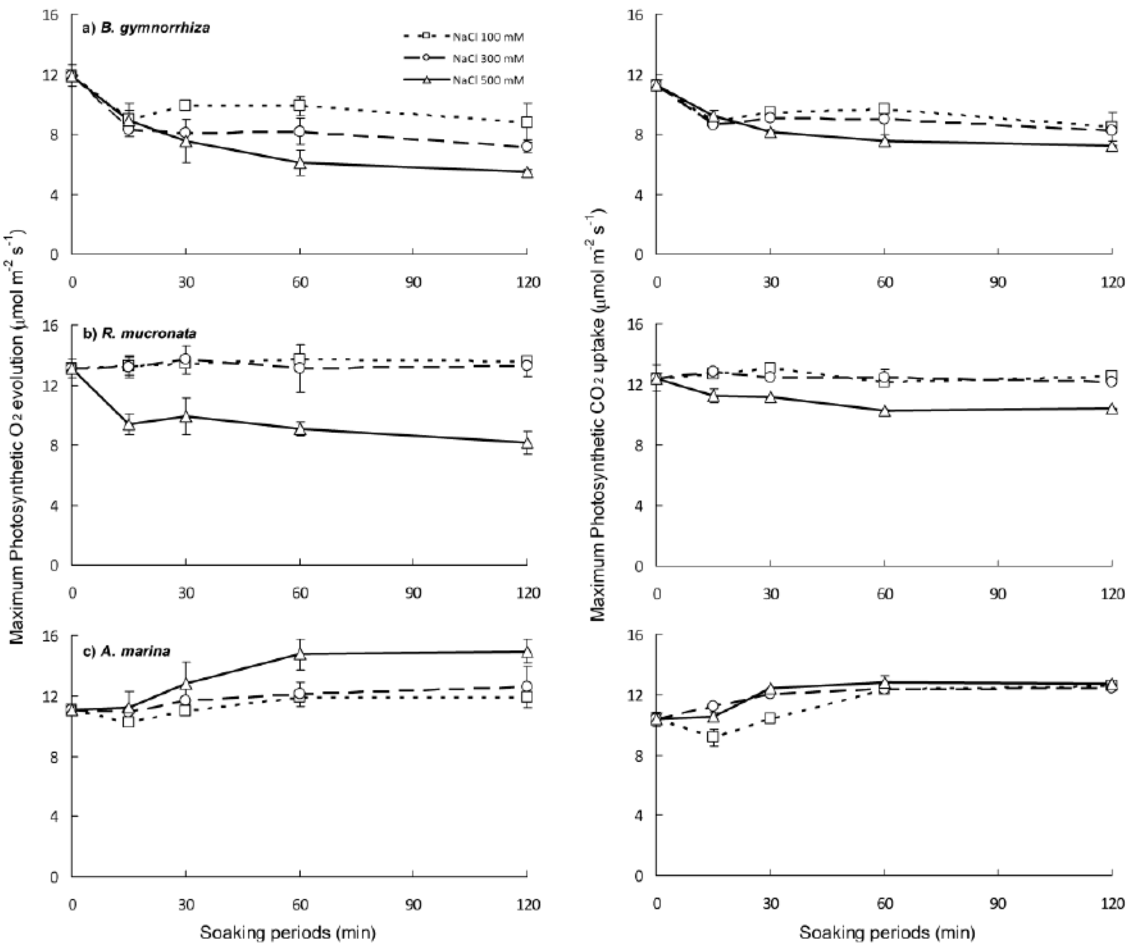

Figure 4 Effects of soaking periods and NaCl concentrations on maximum photosynthetic O_2 evolution and CO_2 uptake in mangrove species. Values are means $\pm$ SD (n=3-4
275 plants).

Short-term impacts of soaking periods and NaCl concentrations to photosynthetic O_2 evolution and CO_2 uptake of mangrove seedlings from East Sumatera Coastline of Indonesia

Abstract. We clarified the photosynthetic performance of different mangrove zonation species (*Avicennia marina*, *Rhizophora mucronata*, and *Bruguiera gymnorrhiza*) under a combination of salinity and soaking stress by using a liquid-phase O_2 electrode combined with CO_2 optodes simultaneously. Photosynthesis O_2 evolution and CO_2 uptake for each mangrove seedlings showed different responses with increasing the soaking period and NaCl concentration. Among three mangrove species, photosynthetic performance in *B. gymnorrhiza* was decreased significantly as compared to the other tested species. On other side, photosynthetic performance of *A. marina* was uniquely increased with prolongation the soaking period and NaCl concentration. Our results showed that *A. marina* maintained the high photosynthetic rate even under the soaking condition. *R. mucronata* had an intermediate response to NaCl concentration during the soaking periods.

Keywords: CO_2 uptake, O_2 evolution, mangrove, photosynthetic rate, salinity, soaking tolerance.

Commented [HK1]: Add description of the problem to be solved by this research

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Commented [HK2]: Explain mechanisms that you believe they stand behind these results.

Introduction

Mangrove is a major and unique coastal ecosystem in tropic area. They have a higher carbon fixation capacity than terrestrial forests [1], adaptation ability under abiotic stress [2], and specific habitat zonation [3]. Mangroves, which thrive luxuriantly in tidal saline wetlands, are especially adapted to salinity and submerged stresses [4].

Belong to the C_3 plant, mangroves also can be classified as "seaweed", since it can grow in high salinity and submerge conditions, whereas C_3 plants could not survive [5]. Furthermore, we use term "soaking condition" to reflect the complete submerged condition whereas the leaves usually immersed in water column [6]. During soaking condition, the chances of plant to fix carbon and capture oxygen are restricted. This situation is worsened due to the irradiance available to sustain underwater photosynthesis for survival is drastically reduced [7].

In recent decades, many workers have been interested in understanding how stress limits mangrove photosynthesis [4] [8] [9]. However, there are relatively few studies on the combined effects of salinity and soaking conditions in mangrove photosynthetic performance [10].

Species differences in mangrove responses to the interactive effects of some stress conditions might explain important differences in mangrove forest structure [11]. *Avicennia marina, Rhizophora mucronata* and *Bruguiera gymnorrhiza* are three dominant mangrove species in East Sumatera coastlines, Indonesia. Based upon illations made from mangrove zonation, these 3 mangrove species appear to differ in their sensitivity to salinity and soaking on ion concentration, tissue water potential and chloroplast [12]. However, the photosynthetic performance between these mangrove species in responses to salinity and soaking conditions have not been well known. The

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mangrove photosynthetic responses as combined soaking-salinity effects could be useful to clarify the mangrove zonation pattern [13].

The estimation of mangrove photosynthetic gas exchange has been evaluated either by O_2 evolution or CO_2 uptake [14] but was limited under soaking conditions as the Infra-Red Gas Analyser is sensitive to water immersion [15]. The simple and stable measurement of mangrove leaf O_2 evolution and CO_2 uptake simultaneously under aqueous conditions have been held using the leaf-disc O_2 electrode with CO_2 optodes sensor [16].

The objective of this research was to investigate the impacts of soaking periods and NaCl concentrations to photosynthetic O_2 evolution and CO_2 uptake of three mangrove species, i.e., *A. marina*, *R. mucronata* and *B. gymnorrhiza*. The photosynthetic responses from each mangrove species will be compared with their specific zonation.

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Materials and Methods

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Plant Materials

Mangrove propagules were collected from East Sumatera Coastline of Indonesia, *A. marina* propagules were collected from Banyuasin Peninsula (02^0 11' S, 104^0 53' E) while *B. gymnorrhiza* and *R. mucronata propagules* from Galang Island (0° 45' N, 104° 15' E). The propagules were initially grown in the greenhouse. After 5 months, seedlings with the fully developed healthy leaves were subjected to treatments. Four levels of soaking periods treatment (15, 30, 60, and 120 min) were employed in each of the three levels of NaCl concentration treatment (100, 300, and 500 mM). There were no NaCl added and soaked for control leaves.

Soaking Periods (Min)	NaCl Concentration (salinity level)		
	100 mM (low)	300 mM (mid)	500 mM (high)
15	√	√	√
30	√	√	√
60	√	√	√
120	√	√	√

After the soaking and NaCl treatments, the leaf sample is sliced using a safety razor under 50 mM HEPES buffer containing 0.5 mM CaSO4 [17], and transferred into the electrode chamber.

Photosynthetic O_2 evolution and CO_2 uptake

Photosynthetic O_2 evolution and CO_2 uptake of mangrove leaves were measured simultaneously as described in [15]. The measurement was held in a closed chamber using a Clark oxygen electrode polarographic sensor (Hansatech Instruments Ltd., , UK) with a sensor of 'pCO2 mini' optodes system (PreSens GmbH, Germany). All measurements were carried out with 20 mM $NaHCO_3$ as carbon dioxide source at 25°C. A slide projector lamp with a calibrated quantum sensor (Hansatech, UK) was used as photosynthetically active radiation (PAR) source.

Photosynthetic response of mangrove leaves at various PAR levels was maintained in decreasing order from 1000 to 50 µmol m^{-2} s^{-1} by placing projector lamp at various distance to the chamber. For a dark respiration measurement, the electrode chamber was wrapped in two layers of aluminium foil. The photosynthetic O_2 evolution and CO_2 uptake were calculated from the initial slopes of the curves during the periods of apparent linear photosynthetic activity. The maximum photosynthetic rate (P_{max}) as the response of photosynthetic rate to light intensity was calculated by using the rectangular hyperbola model [18] [9].

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Results

The light saturation points of all treatments were commonly at PAR level around 500-1000 μmol photon m^{-2} s^{-1} (Figures 1–3). Under control condition, the maximum photosynthetic oxygen evolution of *A. marina* was lower (11.05 μmol m^{-2} s^{-1}) than *B. gymnorrhiza* (11.92 μmol m^{-2} s^{-1}) and *R. mucronata* (13.10 μmol m^{-2} s^{-1}). However, the maximum photosynthetic rate responses indicated different responses while subjected to variation of soaking periods and NaCl concentrations (Figure 4).

During low (NaCl 100 mM) and mid salinity (NaCl 300 mM) under soaked condition, photosynthetic-light responses of *A. marina* had no significance rate with control. The photosynthetic O_2 evolution and CO_2 uptake in *A. marina* was uniquely increased with increasing the soaking period and NaCl concentration. It means the photosynthetic performance of *A. marina* was better under high salinity rather than control during soaking periods (Figure 1 and 4).

For *R. mucronata*, the soaking periods under low and mid salinity did not affect photosynthetic performance and maximum photosynthetic O_2 evolution and CO_2 uptake significantly (Figure 2 and 4). This result suggested that *R. mucronata* adapted up to soaking condition under moderate salinity well like *A. marina*. Under high salinity, photosynthesis of *R. mucronata* declined rapidly and the maximum photosynthetic O_2 evolution dropped more clearly than CO_2 uptake.

In contrast with *A. marina* and *R. mucronata*, all types of soaking periods affected on decreasing of photosynthetic CO_2 uptake and O_2 evolution in *B. gymnorrhiza* (Figure 3 and 4). Maximum photosynthetic rate of *B. gymnorrhiza* decreased simultaneously during soaking periods along salinity escalation. The lessening of maximum photosynthetic O_2 evolution was higher than maximum photosynthetic CO_2 uptake. Photosynthetic performance of *B. gymnorrhiza* encountered the lowest level compared with other species while exposed to high PAR 1000 μmol photon m^{-2} s^{-1} under high salinity.

Discussion

Understanding potential photosynthetic performances of mangroves to soaking, salinity, and light were substantial role for diagnosing successful mangrove lives. This information bring additional important elucidation of mangrove zonation pattern.

The photosynthetic rate-light performance of each mangroves species under control condition reflected that *A. marina* < *B. gymnorrhiza* < *R. mucronata* (Figures 1-3). Clough [19] found that the net photosynthesis performance to light flux density of *B. gymnorrhiza* was lower than *Rhizophora spp.* In other side, the leaf photosynthetic rate of *A. marina* obtained higher than *B. gymnorrhiza* [20]. However, the result indicated that the photosynthetic pattern of these three mangroves varied while subjected to soaking conditions and salinity escalation.

It was substantial to note that low growth and photosynthetic rate was a consequence of mangrove light relationship [11] [9], especially while interacted with other stressor like salinity [21] and soaking. Generally flooding stressed mangrove leaf seedlings than other organs [22]. In contrast, we found that all combinations of soaking and salinity did not depress the leaf photosynthetic rate-light response of *A. marina* seedlings than other mangrove species (Figure 1). This result was in agreement with Kawamitsu et al. finding [5], which obtained that the photosynthesis performance of *A. marina* was not stress even when seedling plants submerged everyday. *A. marina* root system could filter seawater, allowing fresh water only to relocate to the above-ground plant, hence preserved the leaf photosynthetic apparatus [5].

Similar with *A. marina*, there was no significance effects of soaking conditions on photosynthetic rate reducing at low to middle salinity in *R. mucronata* (Figure 2).

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During low to mid soaking and salinity, the primary productivity of mangrove *Rhizophora* was not change significantly [10] and perfomed best seedlings growth [23]. *Rhizophora* maintained photosynthetic–light respons in the moderate inundation and salinity through high stomatal conductance mechanism [23].

In contrast with *A. marina* and *R. mucronata*, assimilation-light performance of *B. gymnorrhiza* for all salinity levels was higher in non-soaking than soaking conditions (Figure 3). It suggested that soaking was stressful to *B. gymnorrhiza* seedlings. A low growth rate in flooded plants could be caused by the negatif effects of flooding on photosynthesis from the leaf to the plant level [6]. Maximum photosynthetic O_2 evolution and CO_2 uptake of *B. gymnorhiza* under saline soaking periods usually lower than control (non-saline soaking periods) (Figure 4*a*). This result indicated that *B. gymnorhiza* was more intolerant to soaking saline condition than *A. marina* and *R. mucronata*. Seedling of *B. gymnorrhiza* had a relatively low tolerance to soaking [24] and also risky to the oxidant damage due to waterlogging [25].

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Rhizophora in natural habitat was more adaptive to soaking and salinity than *Bruguiera* [12]. Our result also supported that *R. mucronata* had the high maximum photosynthetic performance for both CO_2 uptake and photosynthetic O_2 evolution (Figure 4*b*). *Rhizopora* maintained high photosynthetic rate even under stress condition due to their water use efficiency might increase uniquely with decreasing leaf water potential [26]). *R. mucronata*, ‘‘the intermediate gap phase mangrove species’’ had a role as main plant in tropical coastal area and produced high leaf litter [9].

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Regarding on the maximum photosynthetic rate, we supposed that species differences in mangrove responses to soaking and salinity condition showed distinctions characteristic. Maximum photosynthetic CO_2 uptake and O_2 evolution of *A. marina* had a positive effect under high salinity while increasing of soaking periods (Figure 4*c*).

This study indicated that among of the 3 species, *A. marina* is best adapted to tolerate all salinities and soaking conditions. *A. marina* as pioneer vegetation in mangrove ecosystem adapted to broader habitats than *B. gymnorrhiza* and *R. mucronata*. *A. marina* ability to accumulate and excrete salts might contribute to protecting its photosynthetic performance. This result also in line with the study of Naidoo [12], *Avicennia* maintained low stomatal resistance values and tissue water potentials, then high relative water content in order to adapt well to soaking and saline stress condition.

One potential cause of mangrove zonation is the differential ability of propagules to establish at different soaking condition [27]. Our study suggested that the photosynthetic CO2 uptake and O2 evolution of *B. gymnorrhiza* $<$ *R. mucronata* $<$ *A. marina* by escalation of soaking periods and salinity level seem to be appropriate with mangrove natural zonation in Indonesia.

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Acknowledgments

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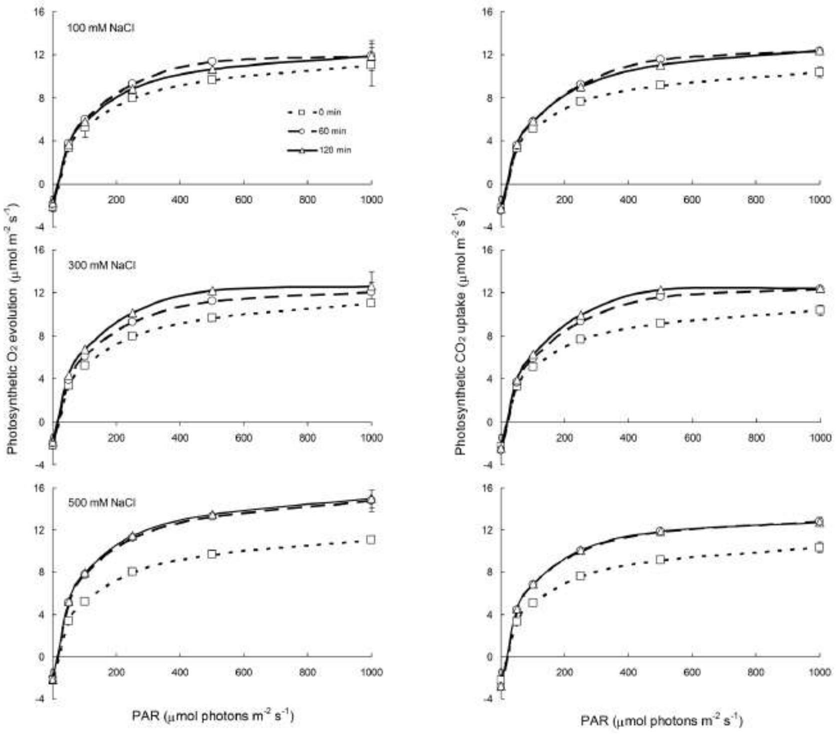

Figure 1 Light response curves for photosynthetic O_2 evolution and CO_2 uptake of mangrove leaves *A. marina* subjected to variation of soaking periods and NaCl concentrations. Control leaves were not soaked (0 min) and no NaCl added. Values are means ± SD (n=3-4 plants).

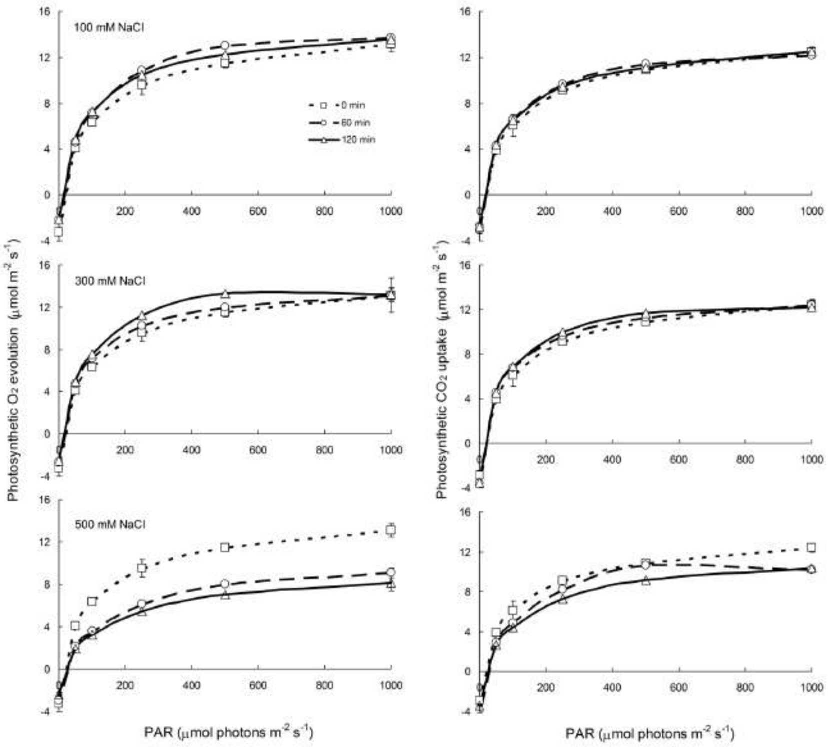

Figure 2 Light response curves for photosynthetic O_2 evolution and CO_2 uptake of mangrove leaves *R. mucronata* subjected to variation of soaking periods and NaCl concentrations. Control leaves were not soaked (0 min) and no NaCl added. Values are means $\pm$ SD (n=3-4 plants).

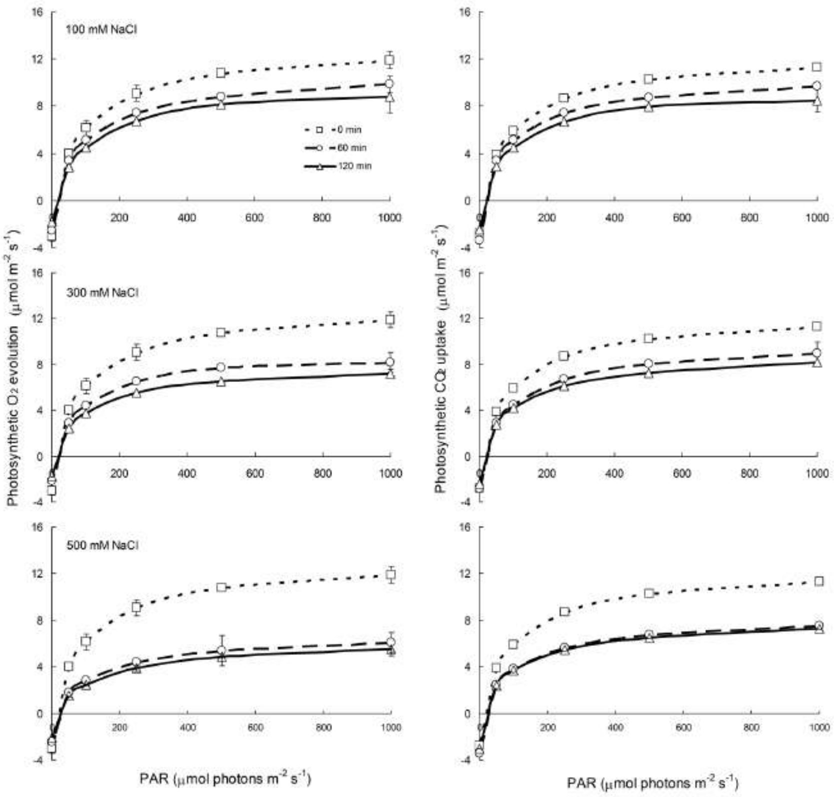

Figure 3 Light response curves for photosynthetic O_2 evolution and CO_2 uptake of mangrove leaves *B. gymnorrhiza* subjected to variation of soaking periods and NaCl concentrations. Control leaves were not soaked (0 min) and no NaCl added. Values are means + SD (n=3-4 plants).

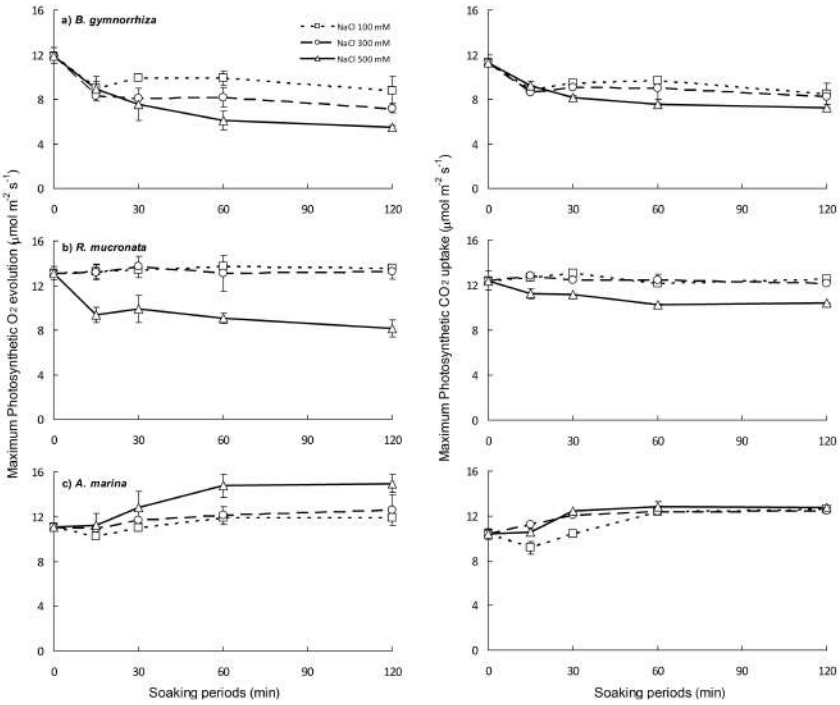

Figure 4 Effects of soaking periods and NaCl concentrations on maximum photosynthetic O_2 evolution and CO_2 uptake in mangrove species. Values are means $\pm$ SD (n=3-4 plants).

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Short-term impacts of soaking periods and NaCl concentrations to photosynthetic O_2 evolution and CO_2 uptake of mangrove seedlings from East Sumatera Coastline of Indonesia

Abstract. Many workers have been interested in understanding how stress limits mangrove photosynthesis. However, there are relatively few studies on the combined effects of salinity and soaking conditions on mangrove photosynthetic performance. We clarified the photosynthetic performance of different mangrove zonation species (*Avicennia marina*, *Rhizophora mucronata*, and *Bruguiera gymnorrhiza*) under a combination of salinity and soaking stress by using a liquid-phase O_2 electrode combined with CO_2 optodes simultaneously. Photosynthesis O_2 evolution and CO_2 uptake for each mangrove seedlings showed different responses with increasing the soaking period and NaCl concentration. Among three mangrove species, photosynthetic performance in *B. gymnorrhiza* was decreased significantly as compared to the other tested species. On other side, photosynthetic performance of *A. marina* was uniquely increased with prolongation the soaking period and NaCl concentration. Our results showed that *A. marina* maintained the high photosynthetic rate even under the soaking condition. *R. mucronata* had an intermediate response to NaCl concentration during the soaking periods.

Keywords: CO_2 uptake, O_2 evolution, mangrove, photosynthetic rate, salinity, soaking tolerance.

Introduction

Mangrove is a major and unique coastal ecosystem in tropic area. They have a higher carbon fixation capacity than terrestrial forests [1], adaptation ability under abiotic stress [2], and specific habitat zonation [3]. Mangroves, which thrive luxuriantly in tidal saline wetlands, are especially adapted to salinity and submerged stresses [4].

Belong to the C_3 plant, mangroves also can be classified as "seaweed", since it can grow in high salinity and submerge conditions, whereas C_3 plants could not survive [5]. Furthermore, we use the term "soaking condition" to reflect the complete submerged condition where the leaves usually immersed in water column [6]. During soaking condition, the chances of plant to fix carbon and capture oxygen are restricted. This situation is worsened because the irradiance available to sustain underwater photosynthesis for survival is drastically reduced [7].

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The estimation of mangrove photosynthetic gas exchange has been evaluated either by O_2 evolution or CO_2 uptake [14] but was limited under soaking conditions as the Infra-Red Gas Analyser is sensitive to water immersion [15]. The simple and stable measurement of mangrove leaf O_2 evolution and CO_2 uptake simultaneously under aqueous conditions have been held using the leaf-disc O_2 electrode with CO_2 optodes sensor [16].

The objective of this research was to clarify and understand responses of soaking periods and NaCl concentrations on photosynthetic O_2 evolution and CO_2 uptake of three mangrove species, i.e., *A. marina*, *R. mucronata* and *B. gymnorrhiza*. The photosynthetic responses from each mangrove species will be compared with their specific zonation.

Materials and Methods

Plant Materials

Mangrove propagules were collected from East Sumatera Coastline of Indonesia, *A. marina* propagules were collected from Banyuasin Peninsula (02^{o} 11' S, 104^{o} 53' E) while *B. gymnorrhiza* and *R. mucronata propagules* from Galang Island (0^{o} 45' N, 104^{o} 15' E). The propagules were initially grown in the greenhouse. After 5 months, seedlings with fully developed healthy leaves were subjected to treatments. Four levels of soaking periods treatment (15, 30, 60, and 120 min) were employed in each of the three levels of NaCl concentration treatment (100, 300, and 500 mM). There were no NaCl added and no soaking for control leaves. After the soaking and NaCl treatments, the leaf sample was sliced using a safety razor under 50 mM HEPES buffer containing 0.5 mM $CaSO_4$ [17], and transferred into the electrode chamber.

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Photosynthetic response of mangrove leaves at various PAR levels was maintained in decreasing order from 1000 to 50 μmol m^{-2} s^{-1} by placing a projector lamp at various distance from the chamber. The broad-band light spectrum produced by the lamp was screened with a quantum sensor (model QRT1, Hansatech Instruments Ltd., UK) in order to determine the amount of photosynthetically active radiation (PAR). For a dark respiration measurement, the electrode chamber was wrapped in two layers of aluminium foil. The photosynthetic O_2 evolution and CO_2 uptake were calculated from the initial slopes of the curves during the periods of apparent linear photosynthetic activity. The maximum photosynthetic rate (P_{max})—was calculated by using the rectangular hyperbola model [18] [9]. A simultaneous measurement of O_2 evolution and CO_2 consumption during photosynthesis was essential in order to clarify the mangrove photosynthetic quotient (PQ) as described previously by Ulqodry [16].

Descriptive statistics were used to obtain the difference between means and standard deviations for each treatment on each dependent variable. All data were given as mean ± SD.

Results

The light saturation points of all treatments were commonly at PAR level around 500-1000 μmol photon m^{-2} s^{-1} (Figures 1–3). Under control condition, the maximum photosynthetic oxygen evolution of *A. marina* was lower (11.05 μmol m^{-2} s^{-1}) than *B. gymnorrhiza* (11.92 μmol m^{-2} s^{-1}) and *R. mucronata* (13.10 μmol m^{-2} s^{-1}). However, the maximum photosynthetic rate responses indicated different responses while subjected to variation of soaking periods and NaCl concentrations (Figure 4).

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Discussion

Understanding potential photosynthetic performances of mangroves to soaking, salinity, and light were important for diagnosing successful mangrove lives. This information bring additional important elucidation of mangrove zonation pattern.

The photosynthetic rate-light performance of each mangroves species under control condition was highest in *R. mucronata*, followed by *B. gymnorrhiza* and *A. marina* (Figures 1-3). Clough [19] also found that the net photosynthesis performance to light flux density of *B. gymnorrhiza* was lower than *Rhizophora spp*. However, according to Kawamitsu et al. [20] the leaf photosynthetic rate of *A. marina* was higher than *B. gymnorrhiza*. Furthermore, the result here indicated that the photosynthetic pattern of these three mangroves varied while subjected to soaking conditions and salinity escalation.

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In contrast with *A. marina* and *R. mucronata*, photosynthetic performance of *B. gymnorrhiza* for all salinity levels was higher in non-soaking than soaking conditions (Figure 3). It suggested that soaking was stressful to *B. gymnorrhiza* seedlings. A low growth rate in flooded plants could be caused by the negative effects of flooding on photosynthesis [6]. Maximum photosynthetic O_2 evolution and CO_2 uptake of *B. gymnorhiza* under saline soaking periods were usually lower than control (non-saline soaking periods) (Figure 4*a*). This result indicated that *B. gymnorhiza* was more intolerant to soaking saline condition than *A. marina* and *R. mucronata*. Seedlings of *B. gymnorrhiza* had a relatively low tolerance to soaking [24] and also risked facing oxidative damage due to waterlogging [25].

Rhizophora in natural habitat was more adaptive to soaking and salinity than *Bruguiera* [12]. Our result also supported that *R. mucronata* had the high maximum photosynthetic performance for both CO_2 uptake and photosynthetic O_2 evolution (Figure 4*b*). *Rhizophora* maintained high photosynthetic rate even under stress condition due to their water use efficiency might increase uniquely with decreasing leaf water potential [26]). *R. mucronata*, ‘‘the intermediate gap phase mangrove species’’ had a role as dominant plant in tropical coastal area and produced high leaf litter [9].

Regarding on the maximum photosynthetic rate, we supposed that species differences in mangrove responses to soaking and salinity condition showed distinctions characteristic. Maximum photosynthetic CO_2 uptake and O_2 evolution of *A. marina* were enhanced under higher salinity and increasing soaking periods (Figure 4*c*). This study indicated that among the three species, *A. marina* is best adapted to tolerate all salinity levels and soaking conditions. *A. marina* as pioneer vegetation in mangrove ecosystem adapted to broader habitats than *B. gymnorrhiza* and *R. mucronata*. *A. marina* ability to accumulate and excrete salts might contribute to protecting its photosynthetic performance. This result was also in line with the report of Naidoo [12] that *Avicennia* maintained low stomatal resistance values and tissue water potentials, and high relative water content in order to adapt well to soaking and saline stress condition.

One potential cause of mangrove zonation is the differential ability of propagules to establish at different soaking condition [27]. Our study suggested that the photosynthetic CO^2 uptake and O^2 evolution of *B. gymnorrhiza* < *R. mucronata* < *A. marina* by escalation of soaking periods and salinity level seem to be appropriate with mangrove natural zonation in Indonesia. White et al. [28], Whitten et al. [29] and Suwignyo et al. [30] observed that mangrove in the west of Indonesia showed a spesific zonation as described : (1) *A. marina*, the mangrove pioneer species, growth commonly in low intertidal swamps under high salinity and long soaking period, (2) *R. mucronata*, occupy dominantly in intermediate zone at the mid-tidal level, and (3) *B. gymnorrhiza*, establish commonly on backside land area under short soaking period and low salinity. It is clear that each mangrove species from different zonation respond differently to different soaking periods and salinity levels.

Acknowledgments

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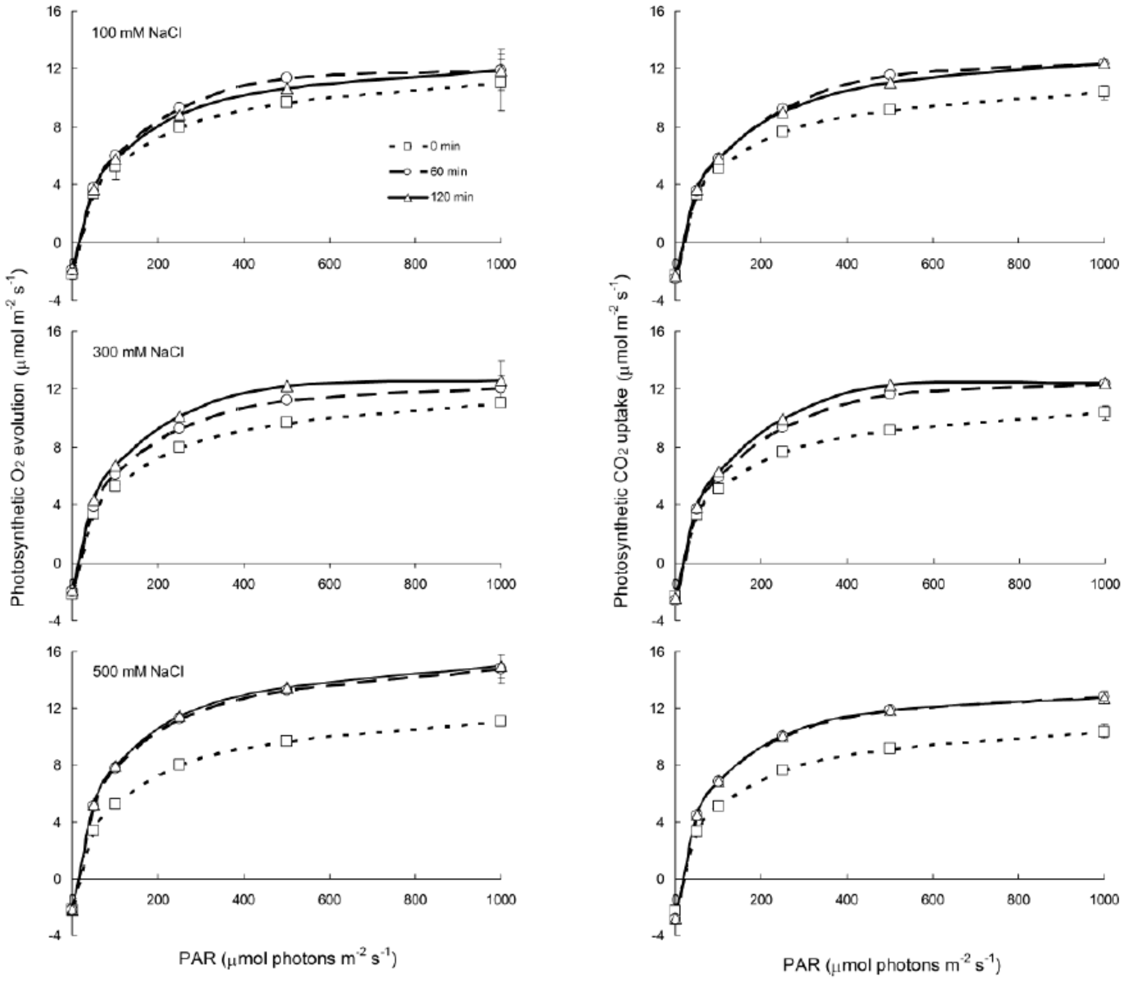

Figure 1 Light response curves for photosynthetic O_2 evolution and CO_2 uptake of mangrove leaves *A. marina* subjected to variation of soaking periods and NaCl concentrations. Control leaves were not soaked (0 min) and no NaCl added. Values are means ± SD (n=3-4 plants).

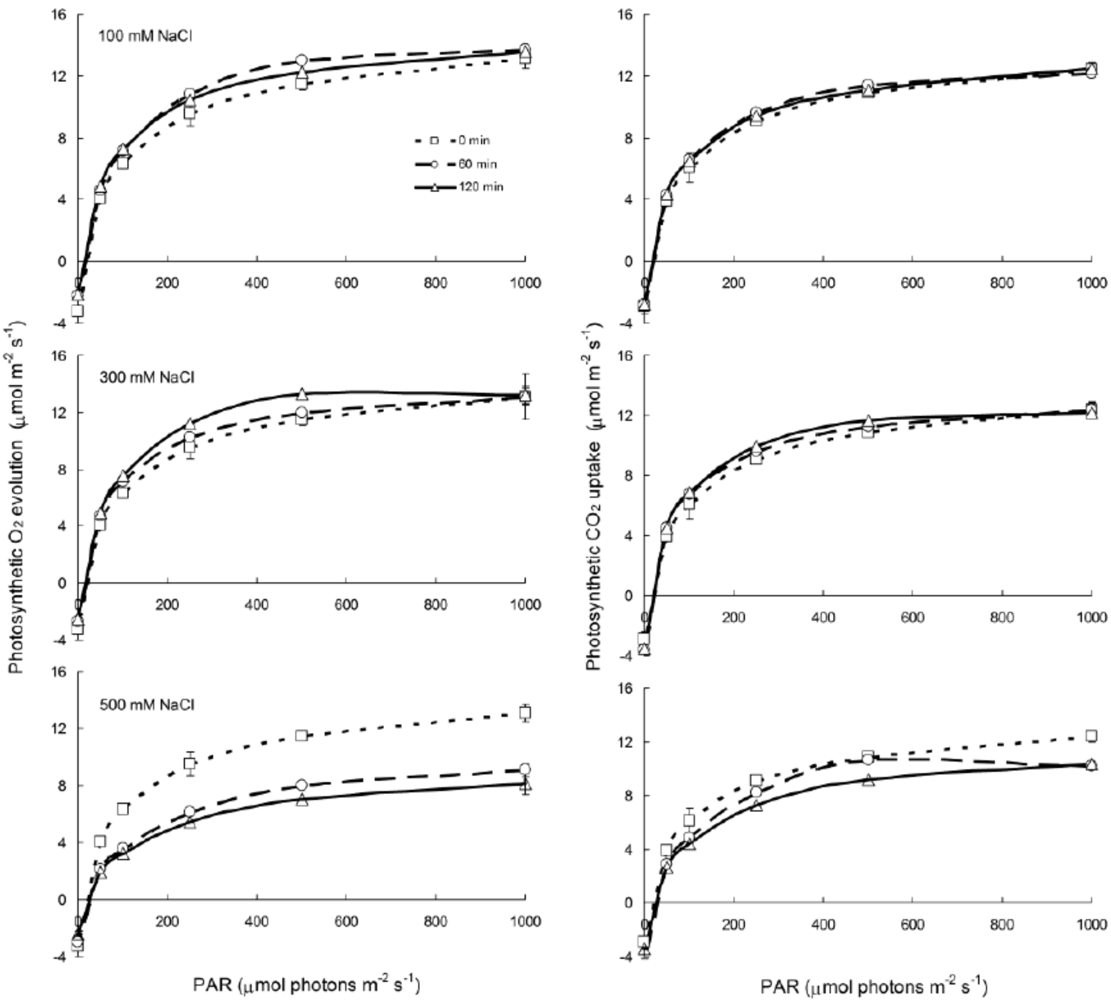

Figure 2 Light response curves for photosynthetic O_2 evolution and CO_2 uptake of mangrove leaves *R. mucronata* subjected to variation of soaking periods and NaCl concentrations. Control leaves were not soaked (0 min) and no NaCl added. Values are means ± SD (n=3-4 plants).

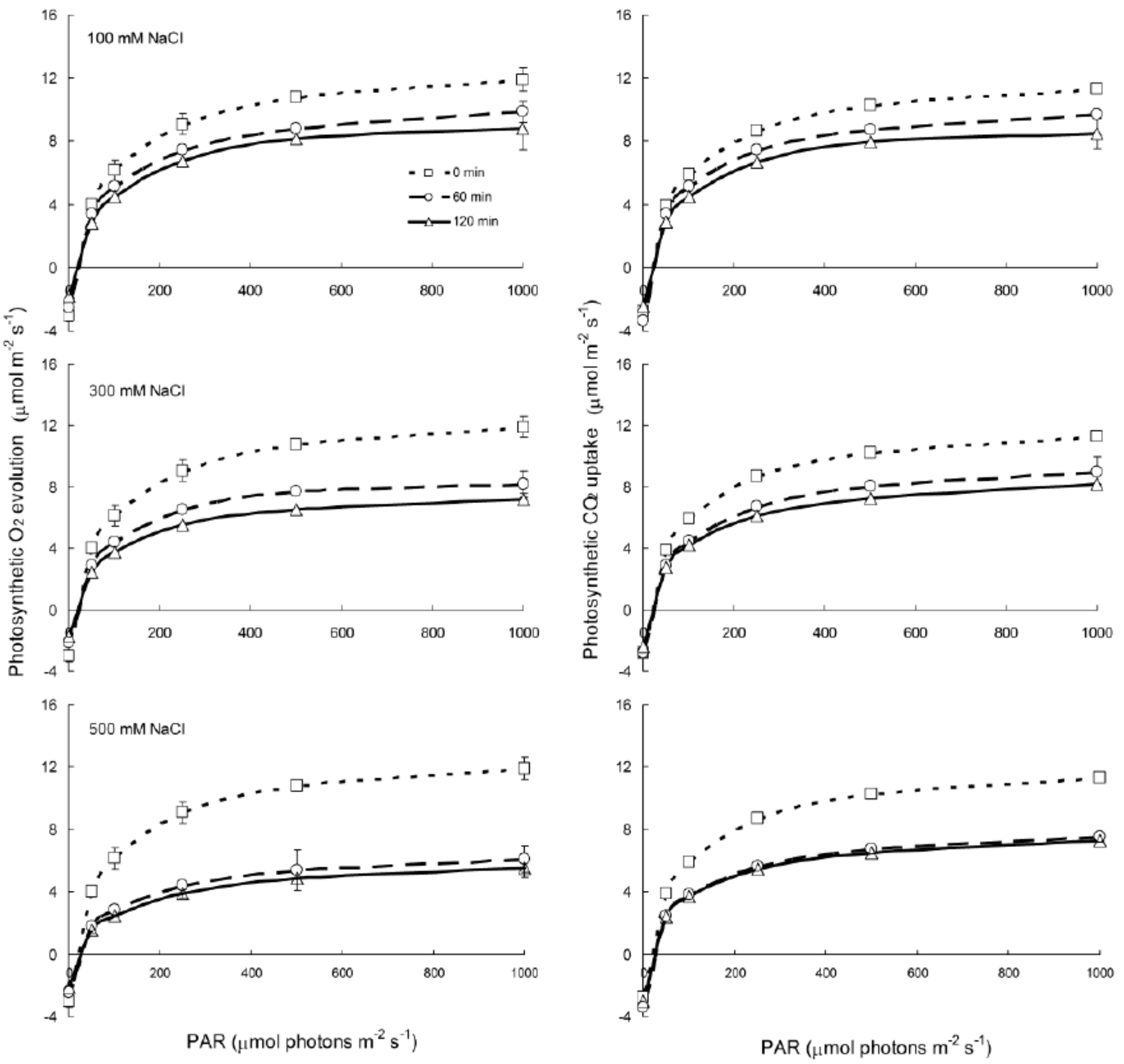

Figure 3 Light response curves for photosynthetic O_2 evolution and CO_2 uptake of mangrove leaves *B. gymnorrhiza* subjected to variation of soaking periods and NaCl concentrations. Control leaves were not soaked (0 min) and no NaCl added. Values are means $\pm$ SD (n=3-4 plants).

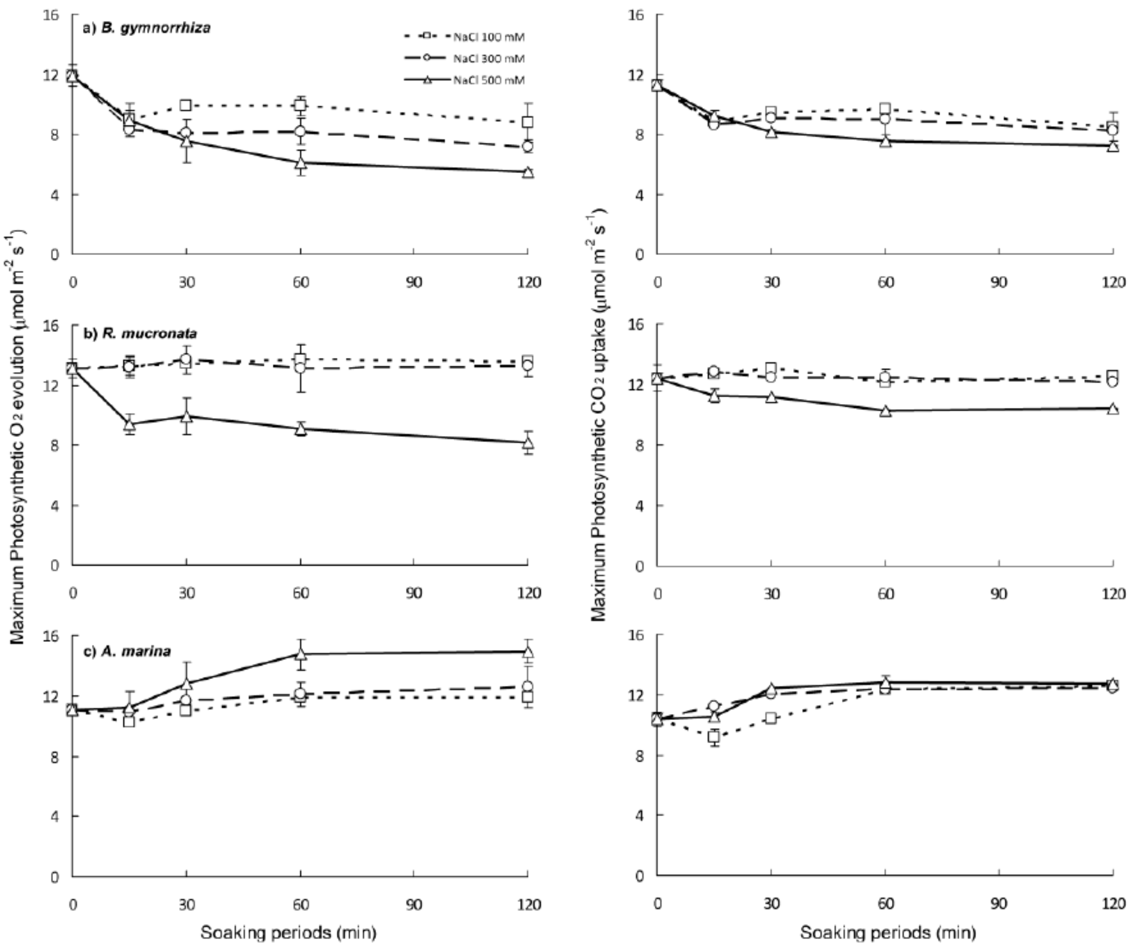

Figure 4 Effects of soaking periods and NaCl concentrations on maximum photosynthetic O_2

280 evolution and CO_2 uptake in mangrove species. Values are means $\pm$ SD (n=3-4 plants).

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Dear Authors,

I am pleased to inform you that, following the satisfactory completion of your revisions in response to the comments of the referees, your paper **CMJS.25.08.18-9480 "Short-term impacts of soaking periods and NaCl concentrations to photosynthetic O_2 evolution and CO_2 uptake of mangrove seedlings from East Sumatera Coastline of Indonesia"** has now been accepted for publication as a **Research Article**. The article will be published in volume 47(1) due in January 2020. Reprints of your paper will be available for download free of charge from our journal website http://epg.science.cmu.ac.th/ejournal/.

On behalf of our Editorial Board, may I thank you very much indeed for publishing your work in our **Chiang Mai Journal of Science** and I sincerely hope that you will consider submitting further articles in the future.

Yours sincerely,

Wasu Pathom-aree

(Asst. Prof. Dr. Wasu Pathom-aree)

Editor-in-Chief