

# CMJS-Shortterm Impacts of Soaking Periods and NaCl

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## Short-term Impacts of Soaking Periods and NaCl Concentrations to Photosynthetic O<sub>2</sub> Evolution and CO<sub>2</sub> Uptake of Mangrove Seedlings from East Sumatera Coastline of Indonesia

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### 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 gymnorhiza*) under a combination of salinity and soaking stress by using a liquid-phase O<sub>2</sub> electrode combined with CO<sub>2</sub> optodes simultaneously. Photosynthesis O<sub>2</sub> evolution and CO<sub>2</sub> uptake for each mangrove seedlings showed different responses with increasing the soaking period and NaCl concentration. Among three mangrove species, photosynthetic performance in *B. gymnorhiza* 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<sub>2</sub> uptake, O<sub>2</sub> evolution, mangrove, photosynthetic rate, salinity, soaking tolerance

### 1. 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<sub>3</sub> plant, mangroves also can be classified as “seaweed”, since it can grow in high salinity and submerge conditions, whereas C<sub>3</sub> 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].

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 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 gymnorhiza* are three dominant mangrove species in East Sumatera coastlines, Indonesia. Based upon illations made from mangrove zonation, these 3 mangrove species might appear to differ in their sensitivity to salinity and soaking conditions [12]. However, the photosynthetic performance between these mangrove species in response to salinity and soaking conditions have not been well 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<sub>2</sub> evolution or CO<sub>2</sub> 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<sub>2</sub> evolution and CO<sub>2</sub> uptake simultaneously under aqueous conditions have been held using the leaf-disc O<sub>2</sub> electrode with CO<sub>2</sub> optodes sensor [16].

The objective of this research was to clarify and understand responses of soaking periods and NaCl concentrations on photosynthetic O<sub>2</sub> evolution and CO<sub>2</sub> uptake of three mangrove species, i.e., *A. marina*, *R. mucronata* and *B. gymnorhiza*. The photosynthetic responses from each mangrove species will be compared with their specific zonation.

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials

Mangrove propagules were collected from East Sumatera Coastline of Indonesia, *A. marina* propagules were collected from Banyuasin Peninsula (02° 11' S, 104° 53' E) while *B. gymnorhiza* 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. 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<sub>4</sub> [17], and transferred into the electrode chamber.

### 2.2 Photosynthetic O<sub>2</sub> Evolution and CO<sub>2</sub> Uptake

Photosynthetic O<sub>2</sub> evolution and CO<sub>2</sub> 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 'pCO<sub>2</sub> mini' optodes system (PreSens GmbH, Germany). All measurements were carried out with 20 mM NaHCO<sub>3</sub> as carbon dioxide source at 25°C.

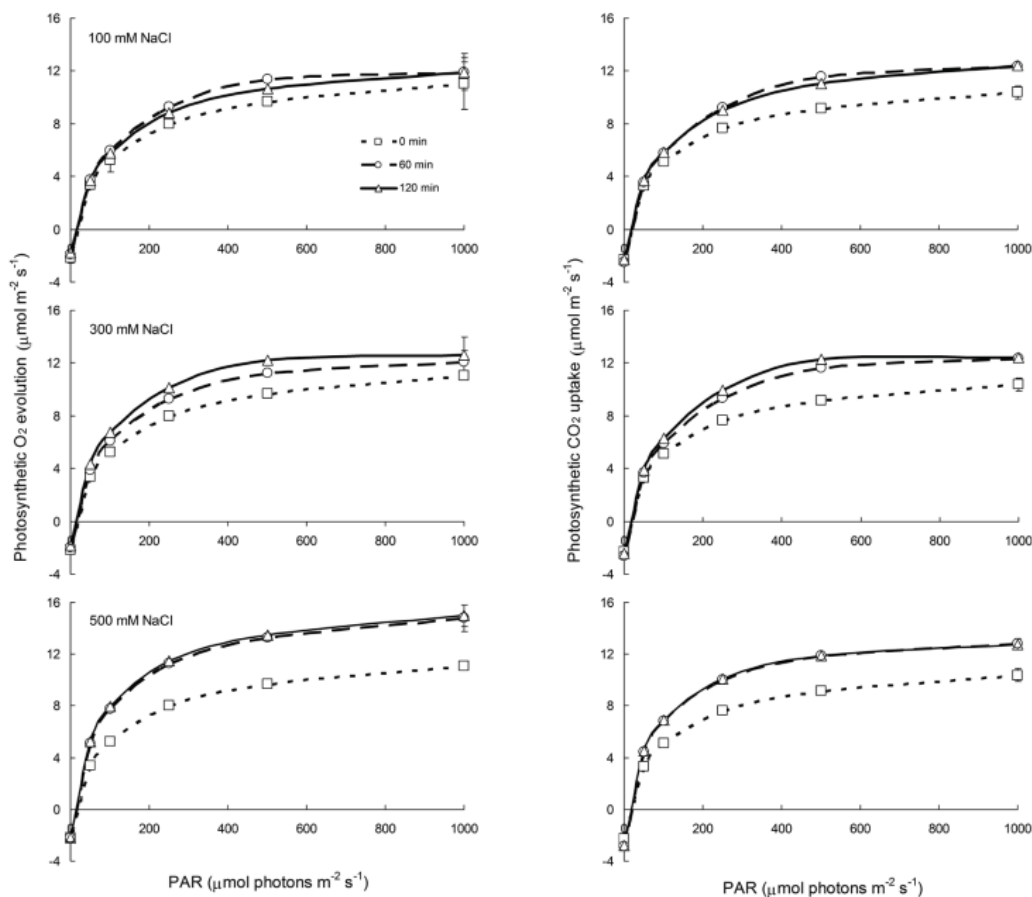
Photosynthetic response of mangrove leaves at various PAR levels was maintained in decreasing order from 1000 to 50 mmol m<sup>-2</sup> s<sup>-1</sup> by placing a projector lamp at various distance from the chamber. The broad-band light spectrum produced by the lamp was screened with a quantum sensor (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<sub>2</sub> evolution and CO<sub>2</sub> 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].

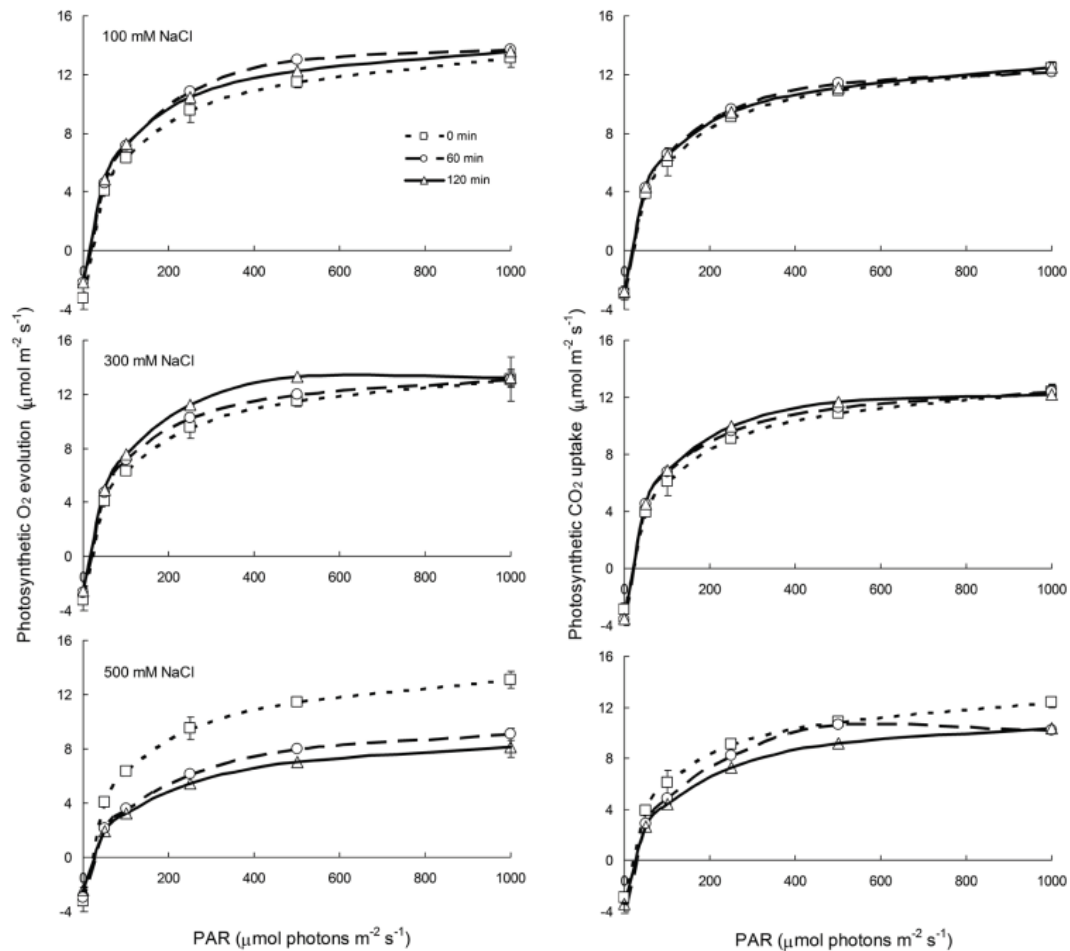
Descriptive statistics were used to obtain the difference between means and standard deviations for each treatment on each dependent variable. All data were given as mean  $\pm$  SD.

### 3. RESULTS

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



**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  $\pm$  SD ( $n=3-4$  plants).

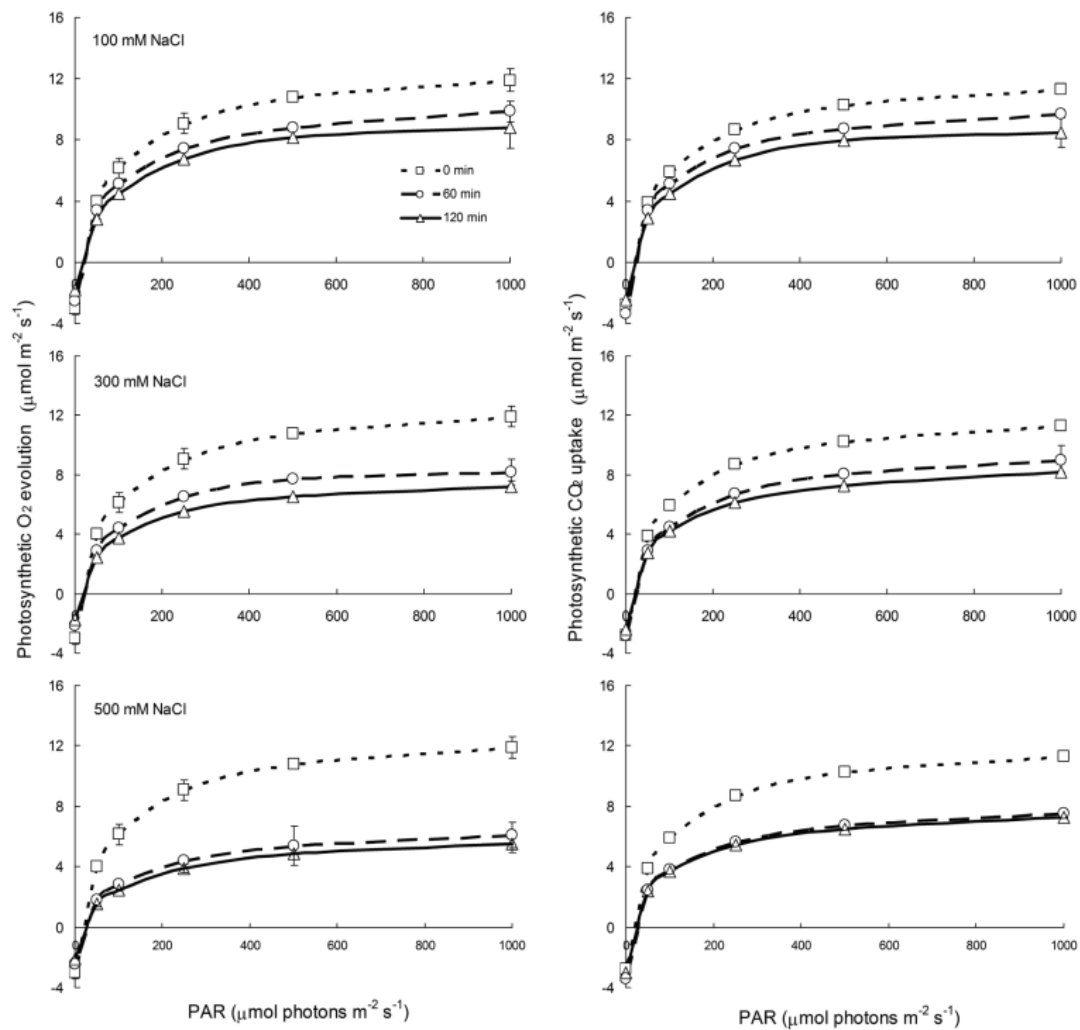


**Figure 2.** Light response curves for photosynthetic  $O_2$  evolution and  $CO_2$  uptake of mangrove leaves *R. mucronata* subjected to variation of soaking periods and NaCl concentrations. Control leaves were not soaked (0 min) and no NaCl added. Values are means  $\pm$  SD ( $n=3-4$  plants).

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. 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.

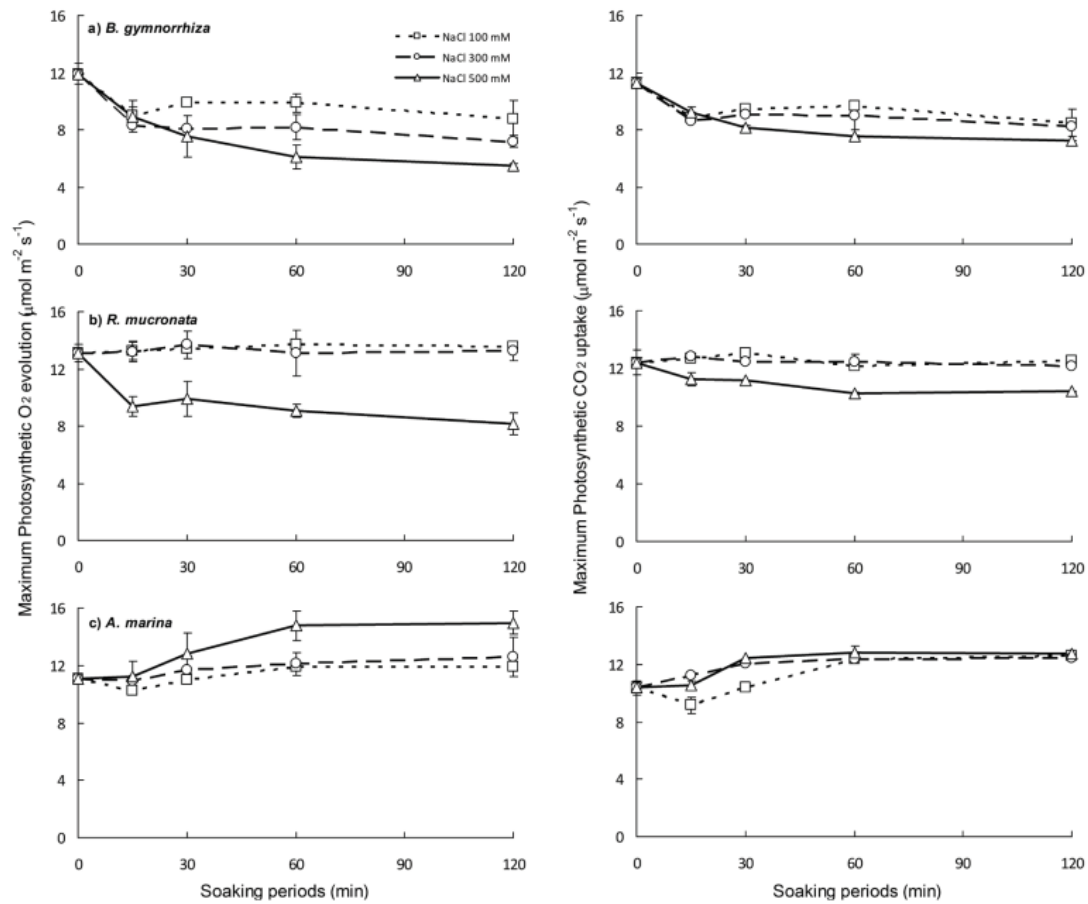




**Figure 3.** Light response curves for photosynthetic O<sub>2</sub> evolution and CO<sub>2</sub> uptake of mangrove leaves *B. gymnorhiza* subjected to variation of soaking periods and NaCl concentrations. Control leaves were not soaked (0 min) and no NaCl added. Values are means  $\pm$  SD (n=3-4 plants).

In contrast with *A. marina* and *R. mucronata*, all types of soaking periods affected on decreasing of photosynthetic CO<sub>2</sub> uptake and O<sub>2</sub> evolution in *B. gymnorhiza* (Figure 3 and 4). Maximum photosynthetic rate of *B. gymnorhiza* decreased

simultaneously during soaking periods along salinity escalation. Photosynthetic performance of *B. gymnorhiza* was lowest compared with other species while exposed to high PAR 1000 mmol photon m<sup>-2</sup> s<sup>-1</sup> under high salinity.



**Figure 4.** Effects of soaking periods and NaCl concentrations on maximum photosynthetic O<sub>2</sub> evolution and CO<sub>2</sub> uptake in mangrove species. Values are means  $\pm$  SD (n=3-4 plants).

#### 4. 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. gymnorhiza* and *A. marina* (Figures 1-3). Clough [19] also found that the net photosynthesis performance to light flux density of *B. gymnorhiza*

was lower than *Rhizophora spp.* However, according to Kawamitsu et al. [20] the leaf photosynthetic rate of *A. marina* was higher than *B. gymnorhiza*. Furthermore, 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 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 (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 every day. *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 [6]. Maximum photosynthetic O<sub>2</sub> evolution and CO<sub>2</sub> uptake of *B. gymnorrhiza* under saline soaking periods were usually lower than control (non-saline soaking periods) (Figure 4a). This result indicated that *B. gymnorrhiza* 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<sub>2</sub> uptake and photosynthetic O<sub>2</sub> evolution (Figure 4b). *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<sub>2</sub> uptake and O<sub>2</sub> evolution of *A. marina* were enhanced under higher salinity and increasing soaking periods (Figure 4c). 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<sub>2</sub> uptake and O<sub>2</sub> 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 specific 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.

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