Decreasing pH affects Seagrass Epiphyte Communities

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Abstract. Increasing CO2 in the atmosphere is affecting marine ecosystems, including seagrass beds. Epiphytes are a component of seagrass ecosystems. Epiphytes make a significant contribution as important primary producers in the food chain. Increasing atmospheric CO2 leads to a decrease in oceanic pH that can result in an unfavorable environment for the epiphytic community. This study was conducted to determine the effect of increasing CO2 on seagrass epiphytic communities and biomass. Using a field experiment, we manipulated dissolved carbon dioxide to 800–1000 ppm in line with the forecast increase in atmospheric CO2 levels in the year 2100. In situ CO2 manipulations were conducted using an open-top mesocosm. The CO2 enrichment was conducted by adding CO2 at a concentration around 800–1000 ppm. This CO2 was injected directly using a pump and hose to the acrylic mesocosm chamber. Epiphyte community structure was affected, with an increase in the abundance of filamentous algae but a decrease in the coralline algae community in the CO2 enriched treatment units. Overall, CO2 enrichment had no effect on epiphyte biomass.

1. Introduction
Global Climate Change is currently the subject of much research as well as serious discussion among researchers. Several studies have conclusively proven the reality of global climate change. The increasing concentration of atmospheric CO2 is a major driver of global climate change, while a rise in mean global temperature is one direct impact.

The rise in atmospheric CO2 is considered to be a critical problem due to its widespread global effect; furthermore, this excess CO2 cannot be reabsorbed through natural ecological processes for several millennia [1]. Over approximately 250 years, atmospheric CO2 concentrations have increased by 40%, from the pre-industrial era level of 280 ppmv (parts per million by volume) to 384 ppmv in 2007 [2]. This increase has occurred due to anthropogenic activities such as industry and deforestation [3]; however, so far, the impacts on land have been relatively low due to absorption of CO2 by the sea [4,5]. The absorption of this additional CO2 is causing a decrease in the pH value of seawater, known as ocean acidification [6].

Since the pre-industrial era, average seawater pH has decreased by 0.1 units from 8.21 to 8.10 [7] and this pollution is projected to further decrease pH by 0.3 to 0.5 units [8] if the concentration of CO2 in the atmosphere reaches 1000 ppmv [9]. In the sea, CO2 dissolves, leading to the formation of HCO3− and CO32− ions, which are used by marine plants as a carbon source for photosynthesis [10].
Seagrass ecosystems play an important role in the cycle of carbon dioxide in the ocean. In these ecosystems, there are plants which live attached to the leaves of seagrasses; these are called epiphytes [11]. Some reviews indicate that CO$_2$ and climate change will have significant impacts on marine ecosystems [12], presumably including seagrass epiphytes.

As an important component in the ecosystem, seagrass epiphytes are likely to be directly and indirectly affected by the increase in CO$_2$ concentration. One study found that increasing CO$_2$ concentration had an impact on the increased growth of filamentous algae epiphyte communities but decreased the growth of epiphyte community of coralline algae in *Thalassia testudinum* [13]. However, another study [14] reported negative impacts on filamentous algae growth with increasing CO$_2$. This research studied the interaction between ocean acidification and the community structure of seagrass epiphytes in an Indo-Pacific seagrass meadow.

2. Methods

2.1. Study Site

This study was conducted in a seagrass bed in Southeast Barrang Lompo island, Makassar, South Sulawesi, Indonesia (05°03’06” LS and 119°19’52.6” BT) at 3 m depth, located close to the fringing coral reef. The seagrass community was dominated by *Enhalus acoroides*. The substrate was composed of sand and coral rubble with some volcano-shaped sand mounds made by burrowing shrimps.

2.2. Experimental Design

The short-term field experiment was carried out from January to February 2015. The experimental design involved manipulating CO$_2$ to decrease pH with a multi-factorial design. This factorial design consisted of three treatment levels: acrylic chambers (mesocosms) with CO$_2$ enrichment, mesocosms without CO$_2$ enrichment, and open plots with no mesocosm or CO$_2$ enrichment. A total of 12 mesocosms (open-top transparent acrylic cylindrical chambers) and 6 open plots (marked with bamboo) were arranged in lines with 6 replicates of each treatment. The cylindrical mesocosms had a cross-sectional area of 0.24 m$^2$ and were spaced at 0.5 m-intervals.

2.3. CO$_2$ Enrichment

The CO$_2$ enrichment was set up to decrease the pH, using a method described in detail in [15]. Gaseous CO$_2$ was injected directly to the acrylic chamber using a pump and hose. The CO$_2$ concentration was controlled and set to approximate the IPCC forecast for 2100, i.e. a pH decreases of 0.3 to 0.5 units [8], through 100% bubbling of compressed air enriched to 800 to 1000 ppm CO$_2$. The chambers were cleaned of epiphytes every once a week. To monitor the quality of the water, water quality measurements were taken daily in all chambers and open plots between 12:00 and 15:00 local time (GMT+8). Water quality parameters measured were pH, temperature, salinity, dissolved oxygen, conductivity and photosynthetically active radiation. A 500 mL water sample was collected from each treatment unit. Salinity, dissolved oxygen, and temperature were measured with a DO Meter (OXY type 1970i), whereas pH was measured with a Multi-parameter WTW (Multi-type 3400i) and photosynthetically active radiation (PAR) was measured with a Light meter (LI-COR type LI-250A).

2.4. Epiphyte Sampling

Epiphyte sampling was conducted after the field experiment. Seagrass leaves were harvested then scanned using a scanner with high resolution (1200 dpi). The scanned results were divided into 4 levels: bottom section (0 – 30 cm), mid-section (30 – 60 cm), top section (60 – 90 cm) and tip section (> 90 cm). A 1 cm$^2$ grid was overlaid and the scan saved as a JPG image. Epiphyte identification and counting was done by observing each 1 cm$^2$ grid square of the resulting image. This step was completed for all parts of the seagrass leaves in every treatment replicate.
2.5. Statistical Analyses
Water quality parameters were tabulated and analyzed in Microsoft Excel to examine the trends and make comparisons between the treatments. Epiphyte abundance was analyzed using the one-way ANOVA function in SPSS 15. When significance was detected (95% confidence level, p < 0.05), a Tukey post-hoc follow-up test was performed.

3. Results
The pH values recorded in the enriched treatment were in the range 5.8–8.8 (mean 7.71), whereas in the non-enriched treatment the range was 6.8 – 8.8 (mean 8.26) and in the non-chamber treatment it was 6.9–8.8 (mean 8.24). The difference between the open and microcosm non-enriched treatments was not significant. Seawater temperature ranged between 27°C and 32°C, while salinity was generally in the range 30–35 ‰ and DO was generally above 6 mg/l; there was no significant difference between any of the treatments. Mean values (±SE) for each parameter and treatment are shown in Table 1.

| Parameters          | n   | Treatment                |
|---------------------|-----|--------------------------|
| pH [ ]              | 30  | 7.71 (±0.12)             | 8.26 (±0.08) | 8.23 (±0.08) |
| Temperature (°C)    | 30  | 29.27 (±0.28)            | 29.35 (±0.29) | 29.32 (±0.3) |
| Conductivity (mS cm⁻¹) | 30  | 47.28 (±0.71)            | 47 (±0.76)    | 47.44 (±0.71) |
| Salinity (‰)        | 30  | 30.9 (±0.5)              | 30.93 (±0.53) | 31.05 (±0.51) |
| Dissolved Oxygen (mg L⁻¹) | 30  | 6.66 (±0.13)            | 6.71 (±0.15) | 6.67 (±0.16) |
| Saturation (%)      | 30  | 95.5 (±2.11)            | 96.06 (±2.05) | 96.18 (±2.34) |
| PAR (µmol m⁻² s⁻¹)  | 30  | 579.5 (±121.9)           | 623.06 (±135.68) | 507.41 (±124.39) |

The epiphyte community comprised 7 main components: encrusting algae, foraminifera, filamentous algae, mollusks, Spirorbis, larvae, and bryozoans. These were grouped into 3 categories: Encrusting, Filamentous and Other (Figure 1).

Encrusting algae appeared to be abundant under the non-enriched treatments, and the count confirmed that abundance was very low under the enriched treatment. Conversely, filamentous algae were visually more noticeable under the enriched treatment, and the number of individuals counted was considerably higher than under the non-chamber treatment. For other epiphyte categories there was no significant difference between any of the treatments.

4. Discussion
This study shows that there were significant between treatment differences between the epiphyte communities. Of these three treatments, encrusting algae appear to dominate the non-enriched treatment and the control. There was a clear distinction between these two epiphytic communities and the enriched treatment where the community was dominated by filamentous algae. It has been suggested that filamentous algae could become dominant on the seagrass Amphibolis antarctica in high CO₂ and high light intensity conditions [14]. In conditions with decreased seawater carbonate, a decrease in the growth of encrusting algal epiphytes on the seagrass Thalassia testudinum has also been reported, accompanied by enhanced growth of epiphytic filamentous algae [13].

The addition of CO₂ under the enriched treatment was continued for ± 45 days, lowering the pH value by around 0.3-0.5 affecting epiphytes communities on seagrass leaves, in particular the growth of filamentous algae which increased over time, while the non-enriched treatment and non-chamber showed no difference. This is consonant with the findings of [16] that short-term CO₂ enrichment can increase photosynthesis, growth, total biomass, and root/shoot carbon and nitrogen ratios of marine plants.

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Encrusting algae growth decreased under the lower pH treatment. This is consonant with other research indicating that encrusting algae is very sensitive to the increase of CO$_2$ that cause a decrease in the availability of CO$_3^{2-}$ ions used in the calcification process. Organisms with a calcareous skeleton will be affected by an increase in atmospheric CO$_2$ due to the concomitant decrease of carbonate ion abundance in seawater [17,18,19].

Some previous studies have reported similar phenomena, with epiphytic encrusting algae community groups disappearing at lower pH values, on average at pH 7.7 [18], with filamentous algae dominating epiphytes on the leaf surface at the lowest experimental pH values [20]. Such phenomena are also affected by stress and limited organismal physiology (e.g. conditions that are not compatible with the environment to which an organism is adapted) [21].

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