Estimation of Metabolic Dynamics of Restored Seagrass Meadows in a Southeast Asia Islet: Insights from Ex Situ Benthic Incubation

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Abstract. Seagrass meadows are vital carbon sinks, but their function is threatened by rapid decline, driving restoration efforts to enhance coastal recovery and carbon removal. The role of restored seagrass meadows as carbon sources or sinks depends largely on organic carbon metabolism and carbonate dynamics. Here, we employed ex situ core incubation to quantify gross primary productivity (GPP), community respiration (R), net ecosystem metabolism (NEM), and net ecosystem calcification (NEC) in restored seagrass (SG) and adjacent bare sediments (BS). SG exhibited significantly higher GPP (26.0 ± 3.4 mmol O_2 m^{-2} h^{-1} *vs* 0.7 ± 1.3 mmol O_2 m^{-2} h^{-1}, $p < 0.01$) and NEM (208.2 ± 22.2 mmol O_2 m^{-2} d^{-1} *vs* 20.1 ± 9.9 mmol O_2 m^{-2} d^{-1}, $p < 0.01$) than BS, indicating enhanced autotrophy and carbon sink potential. SG also exhibited net calcification (10.9 ± 15.7 mmol $CaCO_3$ m^{-2} d^{-1}), while BS showed net dissolution (-2.3 ± 18.8 mmol $CaCO_3$ m^{-2} d^{-1}); however, high NEC variability resulted in no statistically significant difference ($p > 0.05$). These findings suggest that restored seagrass enhances organic carbon sequestration despite variability in carbonate fluxes. Ex situ incubations enable simultaneous measurements of organic and inorganic carbon metabolism, although complementary in situ validation remains essential. Overall,

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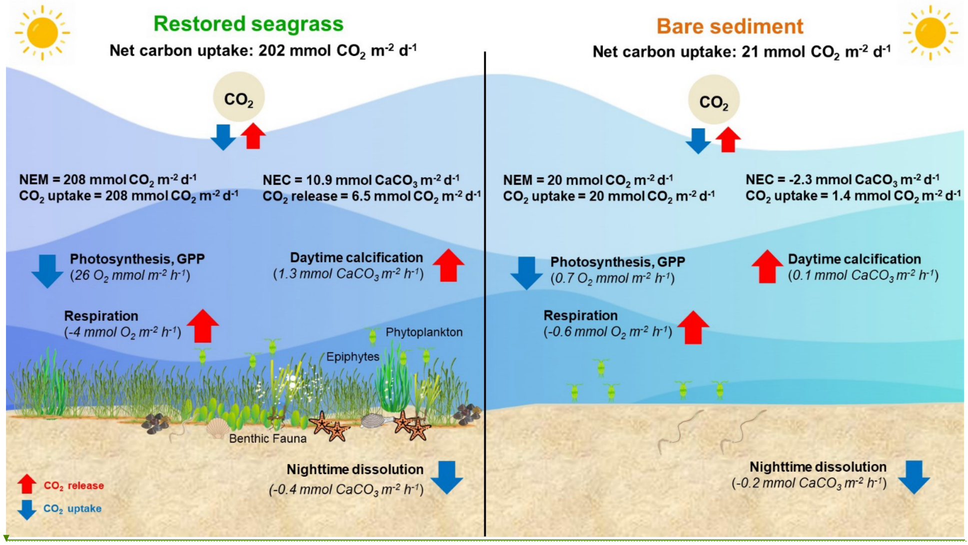
this study highlights the potential of seagrass restoration to strengthen coastal carbon sinks and contribute to climate change mitigation.



Graphical abstract: Illustration of carbon uptake from organic carbon metabolism (GPP-gross primary productivity, R-respiration, NEM-net ecosystem metabolism) and carbonate dynamics (daytime calcification, nighttime dissolution, and NEC-net ecosystem calcification) in restored seagrass and bare sediment. Net Ecosystem Metabolism (NEM).

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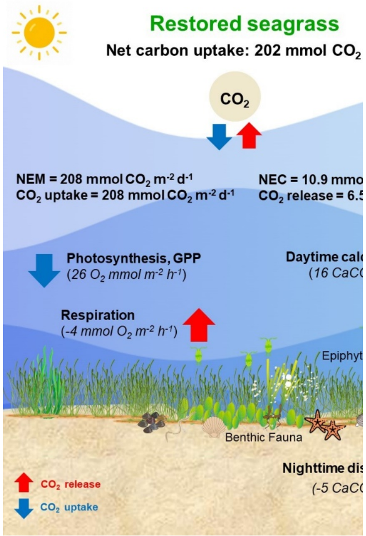

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1 Introduction

Seagrass meadows are marine angiosperms comprising approximately 72 species globally (Short et al., 2011). Although they occupy just 0.1% of the ocean's surface and have limited taxonomic diversity, they are highly productive and ecologically significant ecosystems in the marine environments (Fourqurean et al, 2012; Short et al., 2011). These meadows play essential roles in nutrient and carbon cycling and serve as key habitats for many marine species (Duarte et al., 2010; Fourqurean et al., 2012). Due to their relatively complex structure, seagrass meadows capture and retain organic carbon (C_{org}) in the sediment, making them one of the major carbon reservoirs globally (Duarte et al., 2005; McLeod et al., 2011). Previous estimates suggest that seagrasses account for approximately 15% of the total global carbon sequestered in benthic sediments (Duarte et al., 2013), with burial rates 35 times that of tropical rainforests (McLeod et al., 2011). Nevertheless, carbon storage capacity can vary depending on species-specific traits, geographical location, and environmental conditions (Duarte et al., 2010; Fourqurean et al., 2012).

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In spite of their ecological significance, seagrass meadows have experienced a global decline, driven primarily by human-induced activities such as coastal development, eutrophication, and deteriorating water quality (Orth et al., 2006; Waycott et al., 2009). Since 1980, the global coverage of seagrass has decreased by 110 km² annually, with the rate of decline increasing (Waycott et al., 2009). The loss is frequently associated with increased water column turbidity and epiphytic shading, which reduce the light for seagrass photosynthesis, leading to meadow degradation (Campbell et al., 2003; Orth et al., 2006). Degradation also diminishes their capacity to modify local pH and influence the dynamics of dissolved oxygen (DO) and dissolved inorganic carbon (DIC) (Hendricks et al., 2014). Moreover, the continued loss of seagrass ecosystems raises concerns that vast amounts of previously sequestered carbon could be released back in the atmosphere, converting seagrasses from carbon sinks to carbon sources and intensifying global climate change (Macreadie et al., 2013). The ongoing decline could potentially release up to 299 Tg of carbon annually, contributing roughly 10% of CO_2 emissions associated with anthropogenic land-use changes (Fourqurean et al., 2012).

In response to these challenges, seagrass restoration has emerged as a critical strategy to mitigate environmental degradation, enhance coastal resilience, and address global climate change (Juska and Berg et al., 2022). Protecting and restoring seagrass meadows aligns with international goals like the Paris Agreement, as these ecosystems offer significant potential for long-term carbon storage and climate regulation (Fourqurean et al., 2012). However, despite growing restoration efforts, there remains limited understanding of their success, particularly regarding benthic metabolism and carbon dynamics (Kindeberg et al., 2024). While studies from temperate regions, such as the *Zostera marina* restoration in the Virginia Coast (Rheuban et al., 2014), have provided valuable insights, data from tropical regions — including Southeast Asia, a global hotspot for seagrass diversity — remain scarce (Duarte et al., 2010; Ward et al., 2022; Chou et al., 2023). It represents a critical gap in our knowledge of the impact of restoration efforts on carbon removal and ocean acidification mitigation.

Although there is increasing consensus on the potential of "Blue Carbon" storage in seagrass meadows as a climate change mitigation strategy, the biogeochemical cycling within these ecosystems is complex. Several processes, including ecosystem calcification, anaerobic metabolism, and bioturbation, can counteract net organic carbon (OC) sequestration (Van Dam et al., 2021). These processes regulate local DIC and total alkalinity (TA) budgets, adding complexity to accurately quantifying carbon sequestration (Kindeberg et al., 2024). Overlooking these processes can result in significant overestimates of local carbon sequestration rates and misinterpretations of the role seagrass meadows play in mitigating climate change, potentially leading to inaccurate assessments of their carbon sink capacity (Johansen et al., 2023; Chen et al., 2024; Fan et al., 2024).

Several methodologies were developed to quantify benthic metabolism, which is a crucial component of biogeochemical cycling, including photosynthesis-irradiance curve (Kraemer and Alberte, 1993), the open water O_2 mass balance approach (Odum, 1956; Chou et al., 2023), and aquatic eddy covariance (Berg et al., 2022; Juska and Berg, 2022). While these methods provide important data, they might overlook the complexities of bioturbation, remineralization, and carbonate dynamics (Olivé et al., 2016; Ward et al., 2022; Juska and Berg, 2022). In this study, we aim to address these knowledge gaps by

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quantifying organic carbon metabolism (net ecosystem metabolism, NEM) and carbonate dynamics (i.e., net ecosystem calcification, NEC) in restored seagrass meadows (SG) and adjacent bare sediment (BS) habitats on a Southeast Asia islet, using an innovative ex situ benthic incubation.

2 Materials and Methods

2.1 Study site

The Penghu Islands, located in the southern part of Taiwan Strait (Fig. 1), host a range of seagrass species. Notably, four species have been reported: *Halophila ovalis*, *Halodule pinifolia*, *Halodule uninervis*, and *Zostera japonica* (Yang et al., 2002). The sampling location (23° 38' 18.38" N and 119° 33' 46.48" E) is a restoration meadow dominated by *H. uninervis* and *H. ovalis*. This restoration site encompasses approximately 3 hectares (Allen Coral Atlas, 2020), with seagrass percent cover varying from 20% to 90%. These seagrasses are subtidal, with water depths ranging from 1.7 meters to 4.4 meters. The substrate in this area is composed of carbonate sand. The area supports a diverse community of bivalves (e.g., *Pinna* sp.), gastropods, echinoderms, and various fish species, all of which were observed during the sampling.

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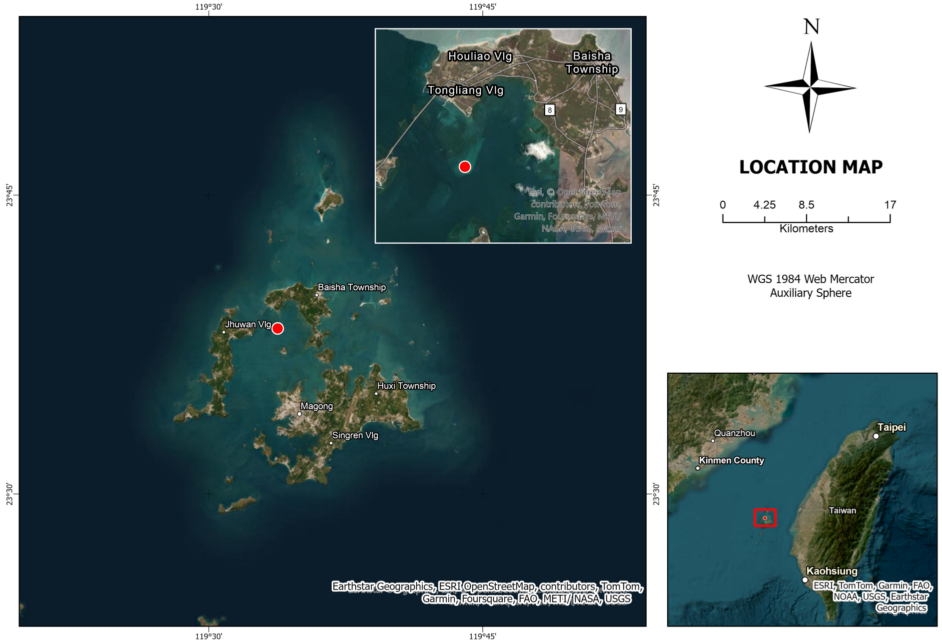

Figure 1: Location map of sampling stations in restored seagrass in Penghu Island, Taiwan (Map created in ArcGIS Pro. Source: Earthstar Geographics, ESRI OpenStreetMap, Contributors, TomTom, Garmin, Foursquare, FAO, METI/NASA, USGS, NOAA).

2.2 Ex situ core incubation system

The ex situ benthic core methodology used in this study was adapted from Chen et al. (2019) (Fig. 2). This approach has been widely employed in various studies to assess nutrient concentrations and benthic metabolism in coastal ecosystems and estuaries (Eyre & Ferguson, 2005; Maher & Eyre, 2011). Typically, the ex situ core incubation involves 150-L treatment tanks containing aerated water. Each tank can accommodate 10 polycarbonate plexiglass cores, 10 cm in diameter and 50 cm in height. The tanks were equipped with magnetic stir bars driven by a centrally located rotating motor fitted with a magnet. Each

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core was sealed with a plexiglass lid containing two ports, one for probe insertion (Eyre & Ferguson, 2005). This method was used to quantify seagrass metabolism, particularly in subtidal systems where in situ measurements are often logistically challenging. While ex situ conditions may differ from natural underwater environments, we carefully designed our setup to closely replicate field conditions, including natural light exposure and ambient temperature, to ensure ecological relevance.

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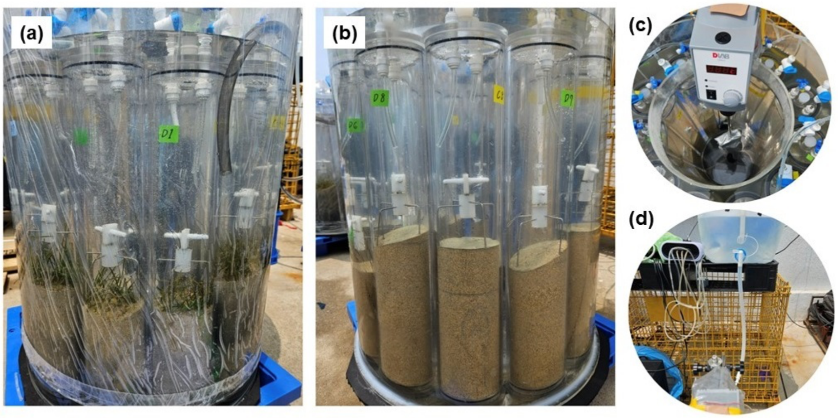

Figure 2: Ex situ benthic chamber setup for measuring metabolic rates and carbonate dynamics in seagrass meadows and bare sediment. The chambers contain seagrass samples (a), while the chambers contain bare sediment (b). Insets show close-ups of the central rotating motor with a magnet setup for water circulation (c), and the setup for continuous seawater supply (d).

2.3 Sediment core collection and pre-incubation

The incubation was conducted on April 12-13, 2024. Twenty intact sediment cores, comprising both seagrass and bare sediment, were collected on-site using the plexiglass tubes. The cores were inserted about 20 cm into the sediment, keeping approximately 1.9 liters of water. Each core was sealed with a gas–tight plexiglass plate at the bottom. The samples were brought back to the incubation site within two hours of collection and allowed to settle for 24 hours. Additionally, 150 liters of water were collected on-site for continuous supply during the experiment.

At the incubation site, the cores were uncovered and placed in 150–liter tanks filled with aerated seawater. They were kept at in situ temperature, exposed to natural sunlight, and continuously recirculated. The stirring rate was controlled to prevent sediment resuspension (Ferguson et al., 2004). The cores underwent a 24–hour pre–incubation period to promote stable sediment profiles. The seagrass composition within the collected cores for ex situ core incubation was dominated by *H. uninervis* and *H. ovalis*. The shoot count of *H. uninervis* ranged from 20 to 40 shoots per 0.008 m², while *H. ovalis* ranged from 2 to 20 shoots per 0.008 m².

2.4 Sample collection and analysis

Following pre-incubation, the cores were tightly closed using a plexiglass lid. Temperature, salinity, and pH were determined using a YSI ProDSS Multiparameter water quality sonde, while DO (mg l^{-1}) was measured with a thermo DO probe. Both probes were calibrated with calibration standards. Measurements were taken at midnight (24:00 h) with 2-hour intervals and ended at noon. Photosynthetically active radiation (PAR) levels were measured using SQ-420X Smart Quantum Sensor positioned atop the incubation tank.

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After measurements, three 150 ml seawater samples were collected separately from the SG and BS cores using a syringe for DIC and pH analysis. The water samples were processed with 60 μL $HgCl_2$ solution to stop any biological activity. DIC analysis was performed using a non–dispersive infrared method with a DIC analyzer (AS-C3, Apollo SciTech Inc.), following the approach of Dickson et al. (2007) and our past studies (Chou et al., 2018; 2021; Fan et al., 2024). For each DIC run, we used certified reference material (Batch no. 206) sourced from A. G. Dickson at Scripps Institution of Oceanography to check for drift and systematic bias. pH values were measured spectrophotometrically in total scale at 25 °C following Clayton and Byrne (1993). Data from DIC and pH, along with actual temperature and salinity, were used to calculate the TA, partial pressure of CO_2 (pCO_2), and aragonite saturation state (Ω_{Ar}) using the Excel macro CO2SYS version 2.1 (Pelletier et al. 2011). The dissociation constants for carbonic acid applied in these calculations were obtained from Mehrbach et al. (1973) and subsequently refined by Dickson and Millero (1987).

2.5 Benthic flux rate calculations

Areal rates of R, GPP, NPP, and NEM were calculated based on changes in DO concentrations, following equation 1 (Eyre et al. 2011). Respiration rates were determined from concentration data collected during the initial dark period (midnight to dawn) (eq. 2). NPP was calculated based on light O_2 flux measurements from dawn to noon (eq. 3). We implemented a 6-hour dark incubation period to ensure oxygen concentrations remained above 80% (Eyre et al., 2002) and a 6-hour light incubation period to prevent oxygen from reaching supersaturated levels (Olivé et al., 2016). Hourly GPP rates were computed as the difference between R and NPP rates (eq. 4). NEM was calculated using equation 5. Positive values indicate autotrophic, while negative values represent heterotrophic.

$$F = [(C_{t1} - C_{t0}) \times V/A]/T \qquad \text{(eq. 1)}$$

Where F = flux rate (μmol m^{-2} h^{-1}), C_{t0} and C_{t1} = concentration in the overlying water at the start and end of the time period (μmol l^{-1}), respectively, V = volume of overlying water in the core (l), A = surface area in the sediment core (m^2), and T = incubation period (h).

$$R = \text{dark } O_2 \text{ flux (negative)} \qquad \text{(eq. 2)}$$

$$NPP = \text{light } O_2 \text{ flux (positive)} \qquad \text{(eq. 3)}$$

$$GPP = NPP \text{ (positive)} - R \text{ (negative)} \qquad \text{(eq. 4)}$$

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$$NEM = (GPP \times 12) - (R \times 24 \text{ h} \times -1) \qquad \text{(eq. 5)}$$

NEC rates (mmol $CaCO_3$ m^{-2} h^{-1}) were estimated from the change of total alkalinity, assuming these changes are only due to $CaCO_3$ precipitation and dissolution (eq. 6) (Roth et al., 2019; Van Dam et al., 2019):

$$NEC = -0.5 \frac{\Delta n\text{TA}}{\Delta t} \; x \; hp \qquad \text{(eq. 6)}$$

Here, ΔnTA = change in nTA (nTA = TA x $SSS_{average}$/SSS) over the Δt (time), h = volume/area, and p = water density. The −0.5 scalar factor was applied to account for the stoichiometric relationship, where 2

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moles of TA produce 1 mole of $CaCO_3$. Day and night incubations (lasting 12 hours) were conducted simultaneously with organic carbon metabolism to obtain daily NEC fluxes. The dark period (midnight to dawn) was used to measure nighttime dissolution, while the light period (dawn to noon) was used for daytime calcification. Alkalinity was measured every 3 hours throughout the incubation period. NEC is positive with TA consumption, indicating $CaCO_3$ precipitation, and negative with TA production, indicating $CaCO_3$ dissolution.

In this study, both hourly and daily rates were reported. Hourly rates allow us to examine diel variations in metabolic processes, while daily rates provide an integrated view of overall carbon dynamics, facilitating comparison with existing literature.

2.5 Statistical analysis

Independent sample T–tests were applied to compare metabolic rates (R, NPP, GPP, NEM, NEC) between SG and BS using SPSS v. 17. Data were subjected to a normality test before performing the analysis. Least–squares linear regression was employed to assess the correlation between changes in DO in the SG and BS. The Mann–Whitney U test was applied for carbonate chemistry analysis due to the non-normal distribution of data.

3 Results

3.1 Water quality and carbonate chemistry

Diurnal patterns of water quality and carbonate parameters for SG and BS during the two-day ex situ core incubation are illustrated in Figs. 3 and 4, respectively. The temperature in both treatments ranged from 22 to 29 °C, while salinity levels spanned from 35 to 36. These values were similar to in situ measurements obtained from the seagrass beds using a CTD profiler. During the daytime (6:00 AM to 12:30 PM), PAR levels ranged from 26 $\mu mol\ m^{-2}\ s^{-1}$ to a peak of 1662 $\mu mol\ m^{-2}\ s^{-1}$, with the highest intensities observed at midday. The average PAR measured 953 $\mu mol\ m^{-2}\ s^{-1}$ on the first day of incubation, increasing slightly to 1026 $\mu mol\ m^{-2}\ s^{-1}$ on the second day. DO saturation levels were more variable in SG than BS, with values ranging from 54% to 224% and 92% to 123%, respectively. DO saturation levels in both treatments followed a diel pattern, with lower nighttime and higher daytime values.

Both *n*DIC (*n*DIC = DIC x $\text{SSS}_{\text{average}}$/SSS) and pH_T displayed greater diurnal fluctuations at SG compared to the BS. At SG, *n*DIC ranged from 1660 to 2118 µmol kg^{-1} (mean ± SD: 1963 ± 153 µmol kg^{-1}), and followed a diel pattern. pH_T ranged from 7.81 to 8.37 at SG (mean ± SD: 7.99 ± 0.2), following the opposite trend to *n*DIC, with values decreasing at night and increasing during the day. At the BS site, these parameters were less variable, with *n*DIC values ranging from 1948 to 2029 µmol kg^{-1} and pH_T from 7.84 to 7.99, with mean values of 1993 ± 27 µmol kg^{-1} and 7.93 ± 0.1, respectively. Similarly, the calculated *n*TA was also more fluctuating in SG than BS, with mean values of 2243 ± 24 µmol kg^{-1} and 2230 ± 24 µmol kg^{-1}, respectively. The calculated *p*CO$_2$ displayed a broader range at SG (142 to 762 µatm; mean ± SD: 510 ± 231) compared to BS (450 to 699 µatm; mean ± SD: 524 ± 82), suggesting a more dynamic carbon cycling potentially driven by seagrass metabolic activity. The mean Ω_{Ar} was higher in SG (3.14 ± 1) compared to BS (2.72 ± 0.4), indicating more favorable conditions for calcification at the seagrass site. Mann–Whitney test on carbonate chemistry revealed no significant distinction between SG and BS (pH_T $p = 0.713$; *n*DIC $p = 0.419$; *n*TA $p = 0.679$; Ω_{Ar} $p = 0.511$).

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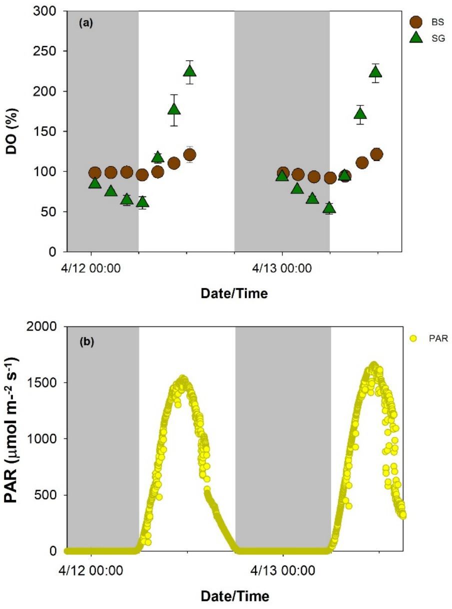

Figure 3: Diurnal pattern of dissolved oxygen (DO, a) in replanted seagrass (SG, green triangle) and bare sediment (BS, brown circle) (n=9, mean ± SD), and photosynthetically active radiation (PAR, b) during the two-day (April 12-13, 2024) incubation.

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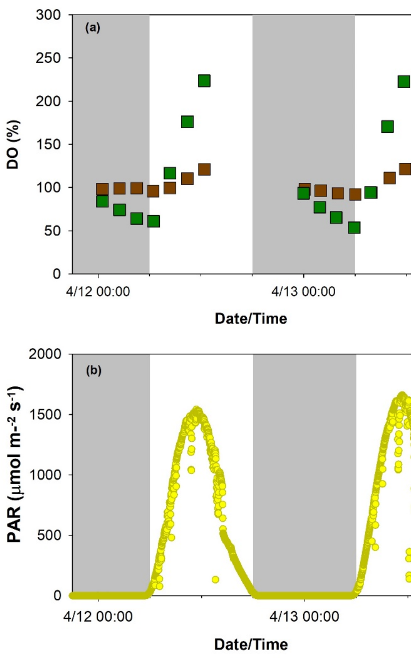

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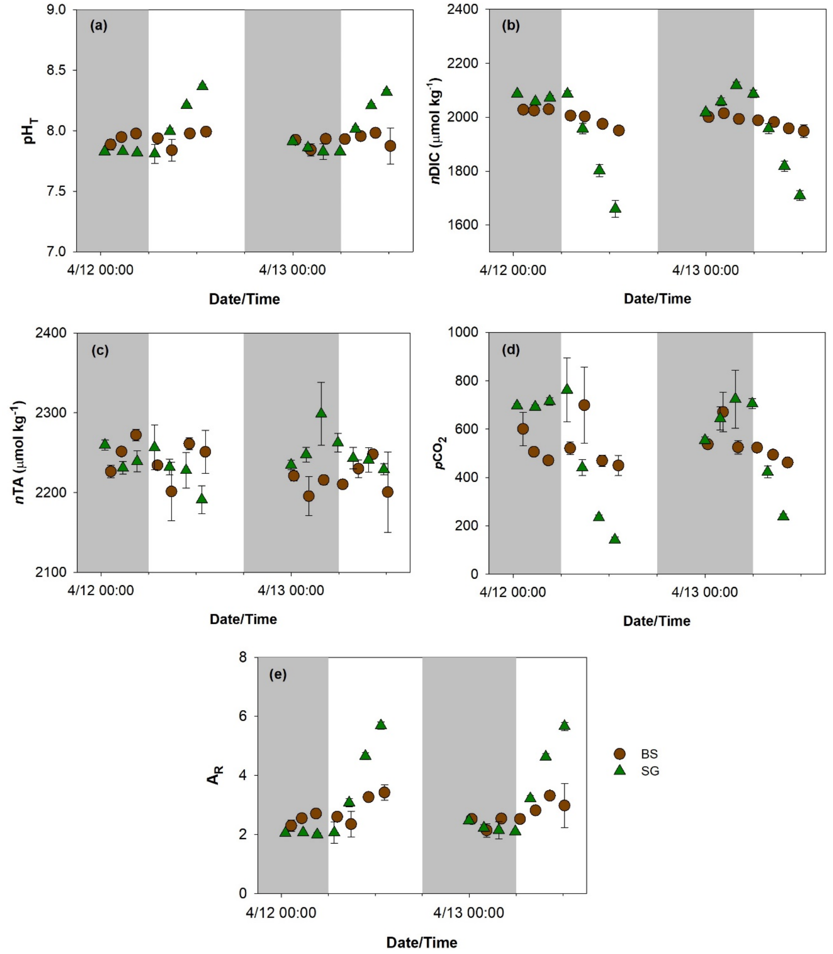
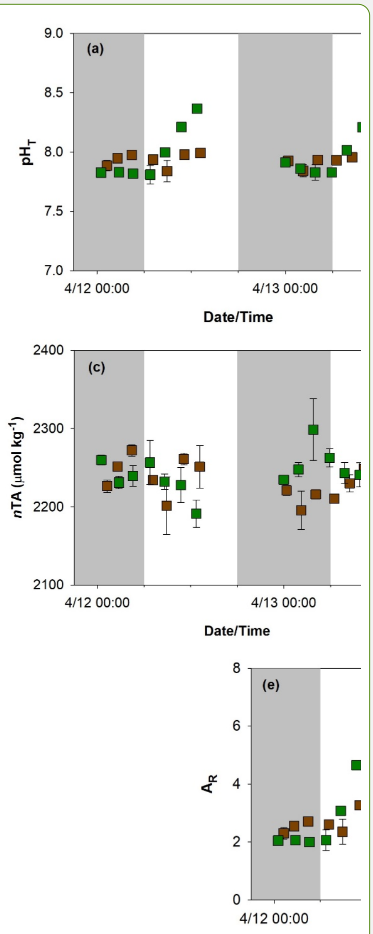


Figure 4: Total scale pH (pH$_T$, a), normalized dissolved inorganic carbon (*n*DIC, b), normalized total alkalinity (*n*TA, c), partial pressure of carbon dioxide (*p*CO$_2$, d), and aragonite saturation state (ΩA$_R$, e) in replanted seagrass (SG, green triangle) and bare sediment (BS, brown circle) during the two-day (April 12-13, 2024) incubation. n=3, mean ± SD.



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3.2 Respiration, gross primary production, and net ecosystem metabolism

Figure 5 illustrates the comparison of metabolic rates (mean ± SD) between SG and BS. The mean respiration rates in SG (-4.3 ± 1.5 mmol O_2 m^{-2} h^{-1}) were significantly higher than in BS (-0.6 ± 0.4 mmol O_2 m^{-2} h^{-1}), by approximately 8-fold difference ($p<0.01$). The mean GPP in SG was 26.0 ± 3.4 mmol O_2 m^{-2} h^{-1}, which is 35-fold higher than in BS (0.7 ± 1.3 mmol O_2 m^{-2} h^{-1}) ($p<0.01$). GPP was always higher than R in both systems, with mean GPP/R ratios of 3.4 and 1.9 in SG and BS, respectively. For NEM, both systems displayed positive values, indicating net autotrophy, with SG being 10-fold higher (208.2 ± 22.2 mmol O_2 m^{-2} d^{-1}) compared to BS (20.1 ± 9.9 mmol O_2 m^{-2} d^{-1}) ($p<0.01$). Both R and GPP in SG and BS increased on the second day of incubation [SG (R: -3.1 vs -5.6 mmol O_2 m^{-2} h^{-1}; GPP: 23.3 vs 24.7 mmol O_2 m^{-2} h^{-1}); BS (R: -0.4 vs -0.81 mmol O_2 m^{-2} h^{-1}; GPP: 2.7 vs 3.1 mmol O_2 m^{-2} h^{-1})], while NEM in SG (218.04 vs 198.4 mmol O_2 m^{-2} d^{-1}) and BS (22.3 vs 17.8 mmol O_2 m^{-2} d^{-1}) showed a slight decrease. However, these changes were not statistically significant.

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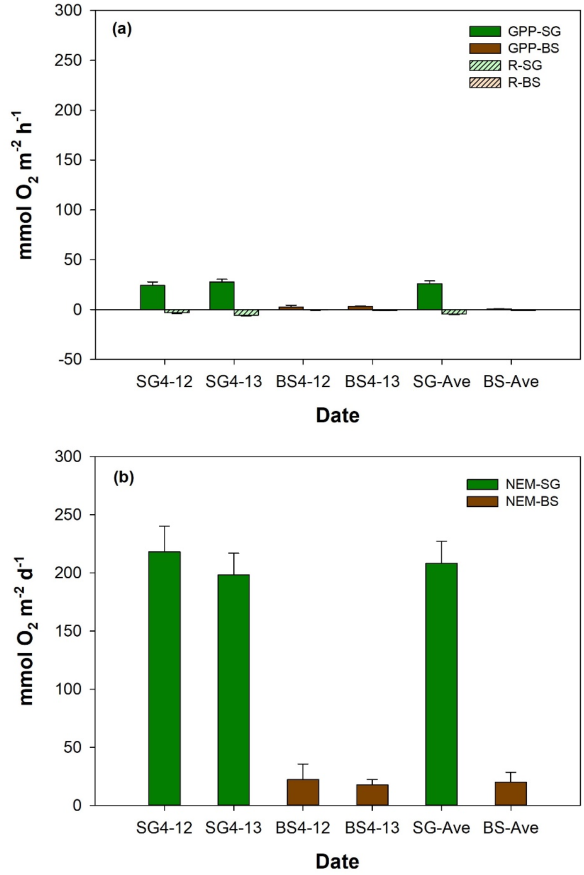
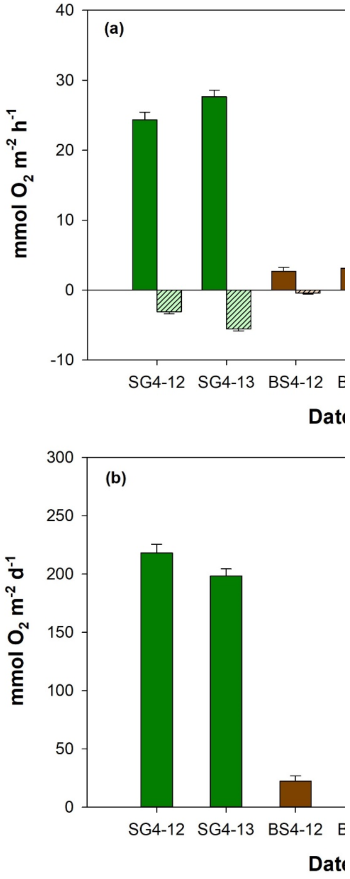


Figure 5: Mean (± SD, standard deviation) values of (a) metabolic rates such as respiration (R), gross primary productivity (GPP), and (b) net ecosystem metabolism (NEM,) of restored seagrass (SG, green bars) and bare sediment (BS, brown bars) in Penghu during the two-day (April 12-13, 2024) incubation (n=9).



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3.2 Calcium carbonate precipitation, dissolution, and net ecosystem calcification

The NEC values (mean ± SD) over a diel cycle for SG and BS demonstrated differences in their overall carbonate dynamics (Fig. 6). Over the two-day incubation period, SG exhibited a net calcifying system with a mean positive daily NEC means (10.9 ± 15.7 mmol CaCO$_3$ m^{-2} d^{-1}), driven by daytime calcification (1.3 ± 1.3 mmol CaCO$_3$ m^{-2} h^{-1}) despite nighttime dissolution (-0.4 ± 0.9 mmol CaCO$_3$ m^{-2} h^{-1}). In contrast, BS supported a net-dissolving system with mean daily NEC (-2.3 ± 18.8 mmol CaCO$_3$ m^{-2} d^{-1}). Mean daytime calcification and nighttime dissolution were 0.1 ± 1.6 mmol CaCO$_3$ m^{-2} h^{-1} and -0.2 ± 0.6 mmol CaCO$_3$ m^{-2} h^{-1}, respectively. Both systems followed a general diurnal pattern, with positive NEC during the day (calcifying) and negative at night (dissolving).

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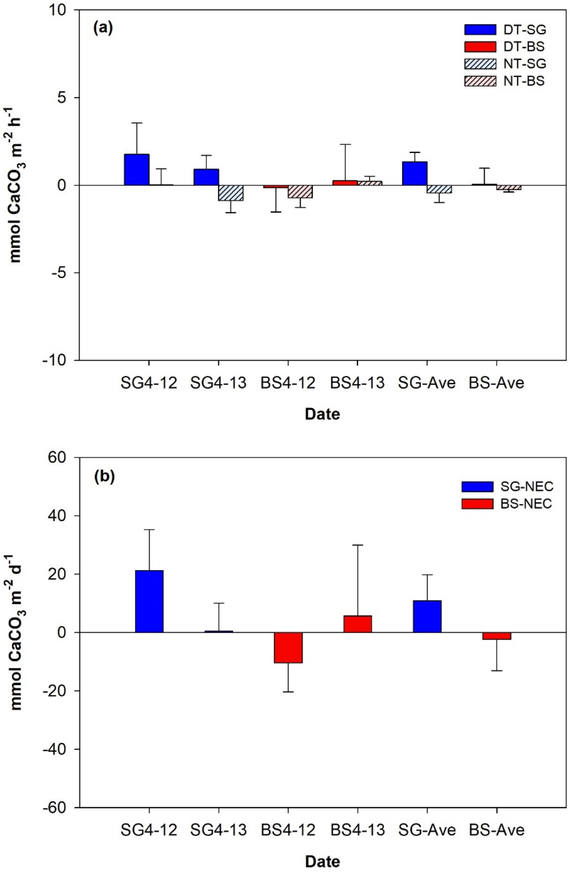
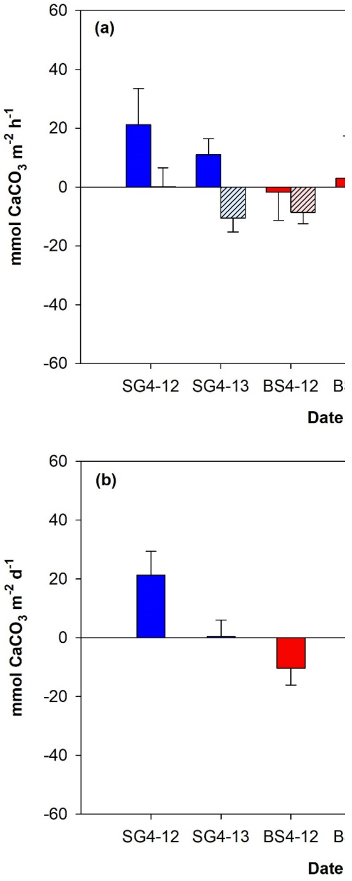


Figure 6: Mean (± SD, standard deviation) values of daytime (DT) and nighttime (NT) calcification (a), and net ecosystems calcification (NEC, b) of restored seagrass (SG, blue bars) and bare sediment (BS, red bars) in Penghu during the two-day (April 12-13, 2024) incubation (n=3).



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4 Discussion

Seagrass meadows are widely recognized as an important blue carbon ecosystem with substantial potential to mitigate anthropogenic CO_2 emissions. Although research on seagrass ecosystems has grown in recent years, significant gaps remain in understanding their carbon dynamics. In particular, the balance of organic and inorganic carbon processes within these systems is not fully understood. Meanwhile, global seagrass coverage continues to decline, which has increased the urgency of restoration efforts (Waycott et al. 2009). Restoring seagrass meadows to enhance carbon sequestration has become increasingly important. Currently, most studies on restored seagrass meadows focus primarily on the burial of particulate organic carbon (Greiner et al. 2013), with far fewer exploring both organic metabolism and carbonate cycling in restored seagrass meadows. Here, we present the first dataset on carbon uptake through metabolic rates and calcification measurements in restored seagrass meadows within tropical regions.

4.1 Restoration of seagrass enhances metabolic rates

The metabolic rates estimated in present study were comparable to those recorded in other seagrass meadows (Table 1). Our GPP in SG was 24% and 37% higher than the tropical and global averages, respectively, but 38% lower than Dongsha Island, Taiwan (Chou et al., 2023). It is also comparable to measurements reported for *H. uninervis* in Tropical Australia (Table 1). Conversely, the R values estimated in this study were roughly half lower than the tropical and global averages (Duarte et al., 2010). Our NEM (214 mmol O_2 m^{-2} d^{-1}) is within the range of previous estimates for tropical seagrass meadows (-477.28 to 484.20 mmol O_2 m^{-2} d^{-1}) and global estimates (-477.28 to 531.63 mmol O_2 m^{-2} d^{-1}). In addition to these global comparisons, our study reveals a clear distinction in metabolic rates (e.g. GPP, R, NEM) between SG and BS. The GPP and R in restored seagrass meadows were 35 and 7 times greater than in BS. The relatively higher metabolic rates in seagrass meadows compared to bare sediments have also been observed in other studies (Table 1). For instance, a two-year-old restored *Halodule wrightii* meadow demonstrated a 13-fold increase in NEM relative to bare sediment (Egea et al., 2023). Similarly, *Posidonia oceanica* exhibited a notable 70-fold increase in metabolic rates compared to bare sediment (Barron et al., 2006). Furthermore, *Zostera marina* exhibits net autotrophy while bare sediments are net heterotrophy (Attard et al., 2019; Chen et al., 2019). Such patterns highlight the fundamental ecological functions restored seagrass meadows play relative to unvegetated/bare sediments. The increase in GPP reflects the enhanced carbon fixation capacity of seagrass meadows, while the elevated R indicates active organic matter decomposition and microbial respiration (Duarte and Krause-Jensen, 2017). According to Duarte et al. (2010), seagrass meadows generally act as autotrophic (NEM > 0) CO_2 sinks when GPP exceeds 186 mmol O_2 m^{-2} d^{-1}, and shift to heterotrophy (NEM < 0) when GPP falls below this threshold. Based on this threshold, our mean GPP for restored seagrass exceeded the value for autotrophy, resulting in a positive NEM which is consistent with their global assessment. The NEM observed in SG was 10 times higher than in BS, suggesting that SG sequesters significantly more carbon than BS. These findings highlight that seagrass restoration significantly boosts metabolic rates and enhances carbon cycling. Given the increasing loss of global seagrass cover, restoration not only boosts ecosystem productivity but also strengthens the ability of coastal systems to remove carbon, thereby contributing to climate change mitigation efforts.

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Table 1. Comparison of metabolic rates from global estimates. GPP and R values are expressed in mmol O_2 m^{-2} h^{-1} units, while NEM in mmol O_2 m^{-2} d^{-1}.

Location	Method	Seagrass Community	GPP	R	NEM	References
Taiwan	Ex situ benthic chambers	Bare sediment	0.74 ± 0.09	0.62 ± 0.09	20.10 ± 2.84	This study
		H. uninervis, *H. ovalis*	25.99 ± 0.96	4.32 ± 0.26	208.21 ± 6.33	
Taiwan	Open water mass balance	*Thalassia, Cymodocea*	42.25 ± 14.42	20.71 ± 7.13	8 ± 61	Chou et al., 2023
Mexico	In situ benthic chambers	Bare sediment	2.13 ± 0.58	0.73 ± 0.16	8.1 ± 10.9	Egea et al., 2023
		2-year *H. wrightii*	13.76 ± 3.35	2.61 ± 0.40	102.4 ± 31.5	
		4-year *H. wrightii*	9.24 ± 2.34	1.60 ± 0.19	72.5 ± 27.9	
		4-year *H. wrightii*	9.34 ± 0.35	2.15 ± 0.25	60.7 ± 4.7	
Sweden	Aquatic eddy covariance and benthic chambers	3-year-old restored seagrass (*Z. marina*)			−5 to −15	Kindeberg et al., 2024
		7-year-old restored seagrass (*Z. marina*)			−21	

Finland	Aquatic eddy covariance	Bare sediment	1.60	0.82	-0.14	Attard et al., 2019
		Z. marina	3.74	1.71	4.17	
Australia	Ex situ benthic	Bare sediment	2.28	1.26	-2.74	Chen et al., 2019
		Zostera sp.	6.94	2.74	7.12	
		Halophila sp.	2.05	1.60	-13.70	
Tropical Australia	Combined methods	*H. uninervis*	23.42 ± 3.67	9.63 ± 4.04	50 ± 53	Duarte et al., 2010
Tropical	Combined methods	All species	21 ± 0.6	9 ± 0.6	24 ± 8	Duarte et al., 2010
Global	Combined methods	All species	19 ± 0.5	8 ± 0.4	27 ± 6	Duarte et al., 2010
Spain	In situ benthic chambers	Bare sediment	0.43	0.22	0.27	Barron et al., 2006
		P. oceanica	7.72	3.18	16.44	

The daily values of R and GPP reported in the literature were divided by 24 and 12, respectively, to calculate the hourly values.

Key drivers of elevated metabolic rates in tropical meadows include greater PAR availability, aboveground biomass, and higher temperatures (Ganguly et al., 2017; Ward et al., 2022). Many tropical species grow near their optimal photosynthetic and physiological conditions (Lee et al., 2007; Koch et al., 2012), efficiently capturing light in shallow, clear waters, which contributes to higher NEP (Ralph et al., 2007). In our study, DO variation corresponds to light intensity (Figs. 3 and 7), suggesting that the elevated GPP observed in seagrass meadows could be driven by higher light intensity. This is likely due to the relatively lower canopy cover of *H. uninervis* and density in SG, which reduces shading within the seagrass. As a result, more light penetrates to the leaves, increasing their photosynthetic surface area and contributing to NEM (Ralph et al., 2007). In contrast, lower respiration rates in the SG area were likely due to the sediment characteristics and organic matter quality in this habitat. The seagrass beds are situated in carbonate-rich sediments, which typically contain less organic matter than siliciclastic or muddy sediments (Belshe et al., 2018; Kindeberg et al., 2018). This limits the availability of substrates for microbial decomposition. Moreover, the organic matter derived from seagrass detritus is generally more refractory and less labile, further reducing its accessibility for microbial breakdown and thereby suppressing heterotrophic respiration (Ren et al., 2024). Although seagrasses are capable of transporting

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oxygen to their belowground tissues via internal aerenchyma (Borum et al., 2006), which can support aerobic respiration, the combined effect of low organic content and poor substrate lability limits microbial activity and oxygen consumption.

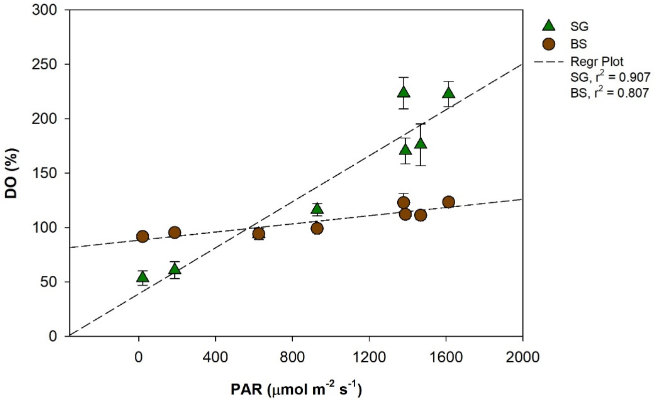
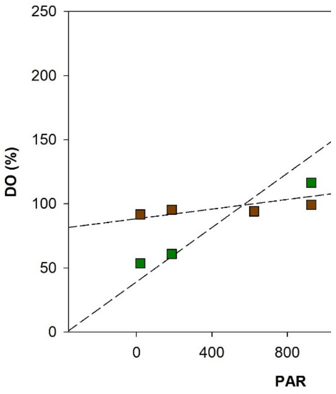


Figure 7: Regression plot between photosynthetically active radiation (PAR, μmol m^{-2} s^{-1}) *vs* dissolved oxygen (DO, %) in restored seagrass (SG, green triangle) and bare sediment (BS, brown circle). Error bars represent standard deviation (SD).

Several studies indicate that restored seagrass can achieve primary productivity and carbon sequestration levels comparable to natural meadows, although recovery depends on the extent of degradation, restoration success, and site-specific habitat conditions (Oreska et al., 2017; Marbà et al., 2015). For example, long-term research in Florida Bay demonstrated that sediment carbon sequestration rates and plant biomass took nearly a decade to match those of natural meadows (Greiner et al., 2013). The ability of restored meadows to maintain net autotrophy is crucial for their role as carbon sinks (Kennedy et al., 2010). This is particularly relevant for climate change mitigation strategies, where the conservation and rehabilitation of this ecosystem are recognized as natural climate solutions (Griscom et al., 2017). Nonetheless, a recent investigation on restored seagrass exhibits net heterotrophy, as observed by Kindeberg et al. (2024) in both 3-year and 7-year-old meadows in Sweden. A similar pattern also reported



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in some natural seagrass meadows in Australia (Chen et al., 2019) (Table 1). This discrepancy underscores the variability in seagrass productivity and metabolic processes based on geographical location and environmental conditions, highlighting the need for region-specific assessments to fully understand seagrass ecosystem dynamics. Long-term studies should also consider temporal and annual variations.

4.2 Calcification dynamics in restored seagrass

Our results show that restored seagrass meadows exhibit significantly higher $CaCO_3$ cycling — both formation and dissolution — compared to bare sediments. This corroborates with prior studies, which documented enhanced carbonate dynamics in vegetated habitats relative to unvegetated sediments. For instance, *P. oceanica* and *Thalassia testudinum* meadows have been shown to promote both $CaCO_3$ production and dissolution (Burdige and Zimmerman, 2002; Barrón et al., 2006), with tropical seagrass ecosystems displaying similar patterns (Chou et al., 2021; Fan et al., 2024). Further, our data revealed a typical diurnal pattern, with positive values during daytime (net calcifying) and negative values during nighttime (net dissolving). These findings align with previous estimates, such as those in Florida Bay, which reported similar diurnal calcification dynamics (Yates and Halley, 2006).

The variations of $CaCO_3$ production and dissolution in surface waters and sediment are related to the carbon cycle through photosynthesis and respiration (Yates and Halley 2006). During daylight hours, photosynthesis raises pH and reduces CO_2 levels in the water, creating favorable conditions for calcium carbonate precipitation—a process referred to as light–enhanced calcification (Schneider et al., 2009). We found a significant positive correlation between PAR and *n*TA changes ($r^2 = 0.52$, $p<0.05$), suggesting that increased light availability may enhance calcification by photoautotrophs in restored seagrass areas during the day (Fig. 8). Additionally, our data showed a significant negative correlation between *n*TA flux and NEM ($r^2=0.54$, $p<0.01$), indicating that higher photosynthetic activity (positive NEM) promotes calcification by consuming TA, while lower NEM or net heterotrophy contributes to TA production, likely through carbonate dissolution or anaerobic decomposition (Fig. 9). Similar relationships between photosynthesis and calcification have been reported in marine calcifiers (Mallon et al., 2022), and the influence of epiphytic organisms in promoting calcification during active photosynthesis has been highlighted in seagrass meadows such as *P. oceanica* (Barrón et al., 2006). At night, carbonate dissolution predominates as aerobic respiration produces CO₂ and carbonic acid in sediment porewater (Eyre et al., 2014), lowering carbonate saturation and driving mineral dissolution (Burdige and Zimmerman, 2002; Burdige et al., 2008; Chou et al., 2021; Fan et al., 2024). The degree of dissolution is directly link to the rate of organic matter decomposition, which depends on the quantity of organic matter, its reactivity, and oxygen availability (Anderson et al., 2005; Morse et al., 2006). High shoot density and root biomass in restored seagrass meadows enhance organic matter supply and decomposition in sediment, further driving nighttime dissolution (Holmer et al., 2013).

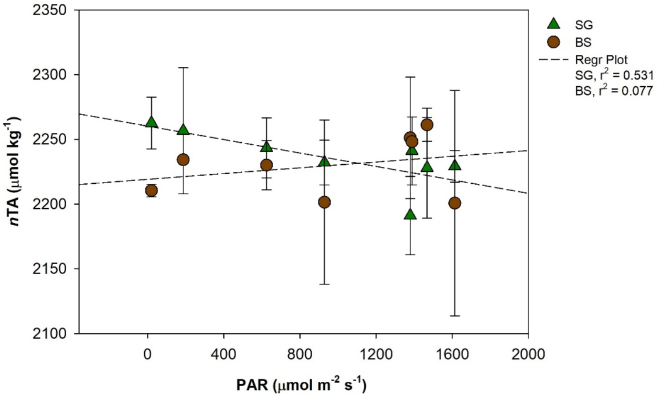

Figure 8: Regression plot between photosynthetically active radiation (PAR, µmol m^{-2} s^{-1}) *vs* normalized total alkalinity (*n*TA, µmol kg^{-1}) in restored seagrass (SG, green triangle) and bare sediment (BS, brown circle). Error bars represent standard deviation (SD).

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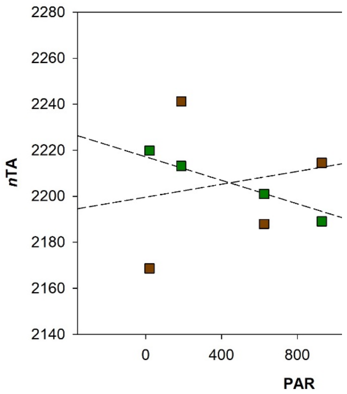

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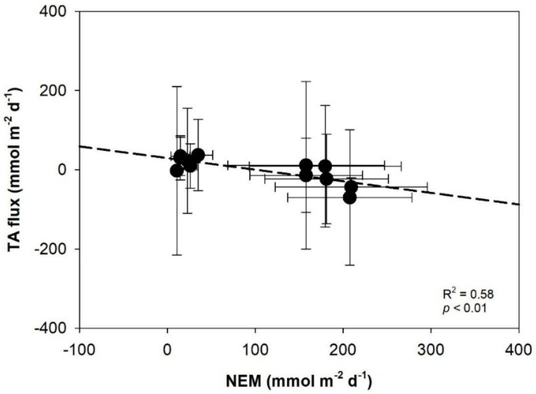
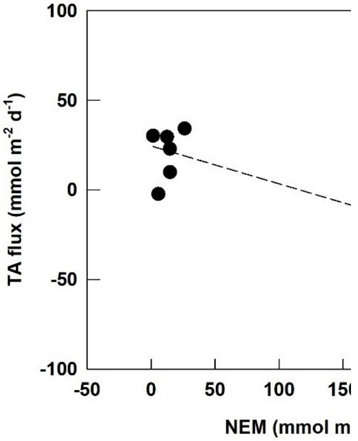


Figure 9: Linear regression showing the relationship between total alkalinity (TA, mmol m^{-2} d^{-1}) flux and net ecosystem metabolism (NEM, mmol m^{-2} d^{-1}) in restored seagrass meadows and bare sediment. Error bars represent standard deviation (SD).

Over cumulative days, our NEC measurements indicate that restored seagrass meadows support overall net calcification, whereas BS supports net dissolution. Our estimates are similar to those from Australia (Walker et al., 1988) and seven times higher than Mediterranean seagrass net calcification rates (Barrón et al., 2006), which are 295 g $CaCO_3$ m^{-2} yr^{-1} (8.8 mmol $CaCO_3$ m^{-2} d^{-1}) and 51 g $CaCO_3$ m^{-2} yr^{-1} (1.40 mmol $CaCO_3$ m^{-2} d^{-1}), respectively. In contrast, our findings are lower than those reported in the Caribbean region of Mexico, where ex situ estimates ranged from 14 to 153 mmol $CaCO_3$ m^{-2} d^{-1} (Enriquez and Schubert, 2014). This highlights the enhanced carbonate production potential in tropical seagrass meadows. A positive net calcification system occurs when $CaCO_3$ precipitation exceeds dissolution within the system (Kleypas et al., 2001; Eyre et al., 2014). Restoration of seagrass meadows provides a substrate for diverse calcifying organisms, including crustose coralline algae, bryozoans, foraminifera, and serpulids, which enhance carbonate production (Beavington-Penney et al., 2005). Epiphytes on seagrass leaves significantly contribute to $CaCO_3$ production, with tropical seagrass meadows typically supporting higher carbonate loads than temperate ones. Reported production rates



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span from 180 g $CaCO_3$ m^{-2} yr^{-1} in Jamaica (Land, 1970) to 2800 g $CaCO_3$ m^{-2} yr^{-1} in Barbados (Patriquin, 1972), underscoring regional variability in seagrass-associated calcification. Moreover, fluctuations in CO_3^{2-} concentrations are crucial in regulating the capacity of calcifying organisms to form $CaCO_3$. Our data reveal a higher mean Ω_{Ar} in SG (3.14 ± 1) compared to BS (2.72 ± 0.4). Seagrass photosynthesis raises pH and Ω_{Ar}, enhancing the calcification of surrounding calcifying organisms (De Beer and Lakrum, 2001). However, the consumption of TA by calcifiers during the calcification process releases CO_2, potentially counteracting pH increases and partially offsetting the net carbon uptake potential of seagrass ecosystems (Alongi et al., 2008; Mazarrasa et al., 2015; Saderne et al., 2019). This highlights the dual role of seagrass restoration in supporting biodiversity and CO_2 uptake while influencing carbonate and carbon flux dynamics. Although the restored seagrass meadow in our study functions as a net calcifying system, TA fluxes between SG and BS showed no significant difference.

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4.3 Net carbon uptake of seagrass restoration

In order to estimate the net carbon uptake potential of seagrass restoration, we applied the photosynthesis-quotient (PQ) of 1 to calculate CO_2 uptake from organic carbon metabolism (Gattuso et al., 1998; Ward et al., 2022). In terms of carbonate dynamics, we applied Φ, as described by Humphreys et al. (2018), to calculate the size of CO_2 source or sink for each system. In the SG system, which is net calcifying, Φ indicates a CO_2 source, with 0.61 moles of CO_2 released into the seawater for each mole of $CaCO_3$ precipitated. In contrast, the BS system, which is net dissolving, Φ represents a CO_2 sink, with 0.65 moles of CO_2 absorbed for each mole of $CaCO_3$ dissolved. These values are comparable to previous findings, which reported a CO_2 flux-to-$CaCO_3$ precipitation ratio of 0.63 (Frankignoulle et al., 1994; Smith, 2013; Mazarrasa et al., 2015). The calculated results show that total carbon uptake from NEM was 208 mmol CO_2 m^2 d^{-1} in SG and 20 mmol CO_2 m^2 d^{-1} BS. For NEC, the carbon release in SG was 6.6 CO_2 m^2 d^{-1}, while for BS, an additional CO_2 uptake was -1.5 mmol CO_2 m^2 d^{-1}. Consequently, the net carbon uptake is 202 and 22 mmol CO_2 m^2 d^{-1} for SG and BS, respectively. Our results demonstrate that the primary productivity of restored seagrass through photosynthesis exceeds the rates of calcification by 31-fold, suggesting that restored seagrass can act as a net carbon sink. However, further assessments are necessary

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to capture temporal variations, as our current measurements are based on daily observations and one season only.

4.4 Limitations of ex situ benthic incubation and future research

We tested the ex situ benthic core incubation approach for restored seagrass meadows, drawing from the existing utilities in some coastal areas and freshwater ecosystems for sulfate and nutrient fluxes (Eyre, et al., 2005, Chen et al., 2019). Overall, the ex situ benthic incubation method provides a significant advantage by measuring both organic and inorganic carbon dynamics simultaneously, addressing a critical gap in previous methods that often overlook carbonate dynamics (Johanssen, 2023). This approach is also useful for assessing seagrass metabolism in subtidal meadows, where collecting data is challenging due to high labor costs and weather conditions. Moreover, some in situ autonomous methods are often expensive and constrained operational periods of only a few weeks due to challenges like sensor error and biofouling (Yates and Halley, 2003; Takeshita et al., 2016). While this approach provides several advantages, one notable limitation is its applicability. Currently, the design is primarily suited for small seagrass, like *H. ovalis*, *H. uninervis*, and *Z. japonica*. It may not be adequate for larger species, like *Enhalus acoroides* and large *Thalassia hemprichii*, due to differences in size and growth characteristics. Moreover, we suggest validating the ex situ results with in situ data to ensure comparability with natural conditions, particularly the effects of light attenuation. Our measurements were obtained under ex-situ conditions in a shallow water column, which likely exposed the cores to higher irradiance than would be encountered in situ at different seagrass depths (2–4 m). While previous research has shown that ex situ and in situ incubations can yield comparable metabolic estimates, supporting the validity of our approach (Maher and Eyre, 2011), we acknowledge the need for future in situ incubations to more accurately capture the natural light environment experienced by seagrass leaves. Future research should integrate ex situ results with in situ data with different geographic and environmental settings to enhance the generalizability of the findings. This will provide a more accurate assessment of seagrass ecosystems' role in global carbon cycling and inform more effective coastal management and conservation practices.

5 Conclusion

This study investigates the organic carbon metabolism and carbonate dynamics of replanted SG compared to BS using the ex situ core incubation method. The results show that SG has higher GPP and NEM, while exhibiting similar NEC, making it a stronger carbon sink than BS. The findings highlight the role of seagrass restoration in enhancing carbon removal and contribute to a growing body of literature that highlights the ecological value of restored seagrass meadows. This study represents the first simultaneous quantitative estimate of the effect of both organic carbon metabolism and carbonate dynamics on carbon sequestration of restored seagrass in Southeast Asia, providing valuable insights into the region's carbon dynamics. We emphasize the need for long–term research on metabolic rates and carbonate dynamics to account for temporal variations and to fully understand the implications of these processes in carbon sequestration. This will also help optimize restoration strategies aimed at maximizing carbon sink potential and mitigating ocean acidification. Furthermore, ex situ benthic incubation proves to be a valuable tool for assessing carbon fluxes in seagrass meadows, particularly those dominated by pioneering species, although further in situ assessments are necessary for comprehensive validation.

Author contribution

WCC and JJC conceptualized the research and spearheaded the implementation. JJC, MBN, HYC, and YLS facilitated sample collection and analysis. MBN and JJC performed the data analysis, drafted the manuscript, and its revision. WCC and LFF reviewed and revised the manuscript. All authors were involved in the finalization of the manuscript.

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Competing interest

The authors declare that they have no conflict of interest.

Data availability

The data supporting the findings of this study are available in the DRYAD repository at https://doi.org/10.5061/dryad.d7wm37qd0 (Natividad et al., 2025).

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