

1 Estimation of Metabolic Dynamics of Restored Seagrass Meadows in a Southeast Asia Islet:

2 Insights from Ex Situ Benthic Incubation

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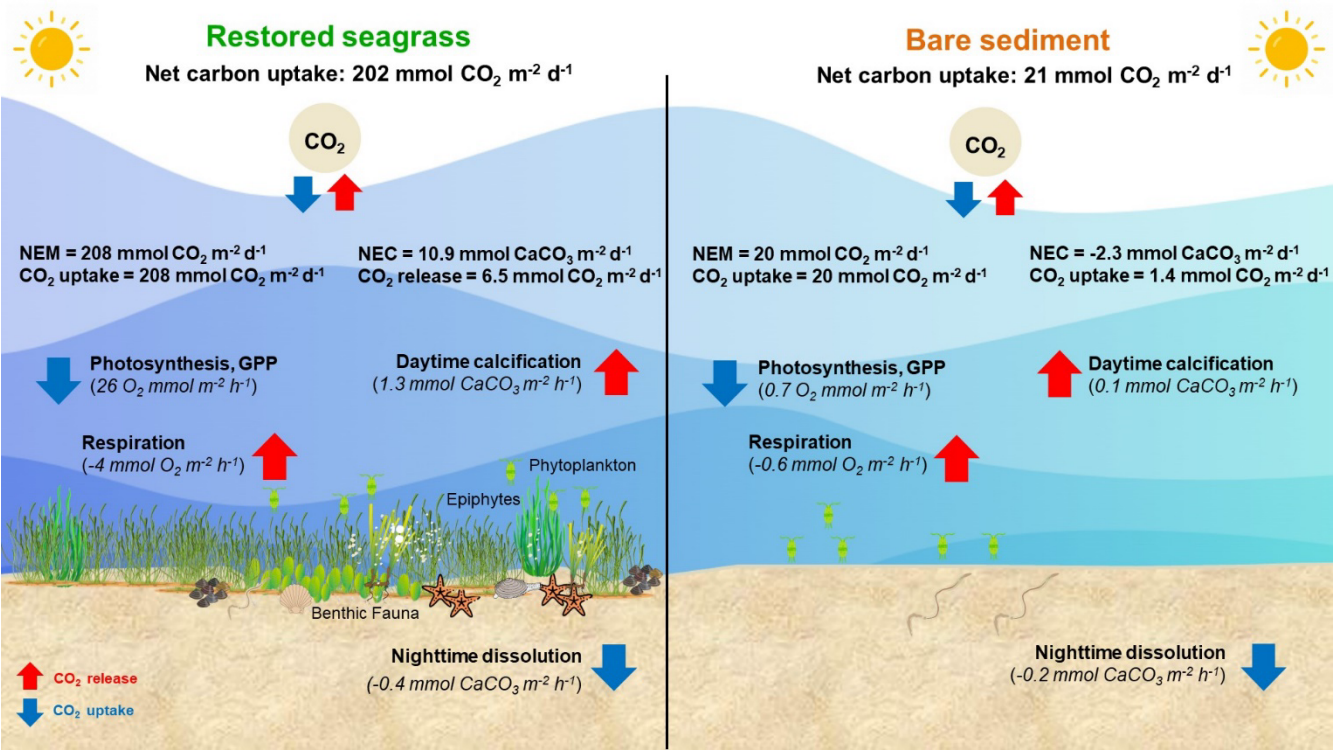
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17 **Abstract.** Seagrass meadows are vital carbon sinks, but their function is threatened by rapid decline,
18 driving restoration efforts to enhance coastal recovery and carbon removal. The role of restored seagrass
19 meadows as carbon sources or sinks depends largely on organic carbon metabolism and carbonate
20 dynamics. Here, we employed ex situ core incubation to quantify gross primary productivity (GPP),
21 community respiration (R), net ecosystem metabolism (NEM), and net ecosystem calcification (NEC) in
22 restored seagrass (SG) and adjacent bare sediments (BS). SG exhibited significantly higher GPP ($26.0 \pm$
23 $3.4 \text{ mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$ vs $0.7 \pm 1.3 \text{ mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$, $p < 0.01$) and NEM ($208.2 \pm 22.2 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ vs
24 $20.1 \pm 9.9 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$, $p < 0.01$) than BS, indicating enhanced autotrophy and carbon sink potential.
25 SG also exhibited net calcification ($10.9 \pm 15.7 \text{ mmol CaCO}_3 \text{ m}^{-2} \text{ d}^{-1}$), while BS showed net dissolution
26 ($-2.3 \pm 18.8 \text{ mmol CaCO}_3 \text{ m}^{-2} \text{ d}^{-1}$); however, high NEC variability resulted in no statistically significant
27 difference ($p > 0.05$). These findings suggest that restored seagrass enhances organic carbon sequestration
28 despite variability in carbonate fluxes. Ex situ incubations enable simultaneous measurements of organic
29 and inorganic carbon metabolism, although complementary in situ validation remains essential. Overall,

30 this study highlights the potential of seagrass restoration to strengthen coastal carbon sinks and contribute
31 to climate change mitigation.
32

33



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

38 1 Introduction

39 Seagrass meadows are marine angiosperms comprising approximately 72 species globally (Short et al.,
40 2011). Although they occupy just 0.1% of the ocean's surface and have limited taxonomic diversity, they
41 are highly productive and ecologically significant ecosystems in the marine environments (Fourqurean et
42 al, 2012; Short et al., 2011). These meadows play essential roles in nutrient and carbon cycling and serve
43 as key habitats for many marine species (Duarte et al., 2010; Fourqurean et al., 2012). Due to their
44 relatively complex structure, seagrass meadows capture and retain organic carbon (C_{org}) in the sediment,
45 making them one of the major carbon reservoirs globally (Duarte et al., 2005; McLeod et al., 2011).
46 Previous estimates suggest that seagrasses account for approximately 15% of the total global carbon
47 sequestered in benthic sediments (Duarte et al., 2013), with burial rates 35 times that of tropical rainforests
48 (McLeod et al., 2011). Nevertheless, carbon storage capacity can vary depending on species-specific
49 traits, geographical location, and environmental conditions (Duarte et al., 2010; Fourqurean et al., 2012).

50

51 In spite of their ecological significance, seagrass meadows have experienced a global decline, driven
52 primarily by human-induced activities such as coastal development, eutrophication, and deteriorating
53 water quality (Orth et al., 2006; Waycott et al., 2009). Since 1980, the global coverage of seagrass has
54 decreased by 110 km² annually, with the rate of decline increasing (Waycott et al., 2009). The loss is
55 frequently associated with increased water column turbidity and epiphytic shading, which reduce the light
56 for seagrass photosynthesis, leading to meadow degradation (Campbell et al., 2003; Orth et al., 2006).
57 Degradation also diminishes their capacity to modify local pH and influence the dynamics of dissolved
58 oxygen (DO) and dissolved inorganic carbon (DIC) (Hendricks et al., 2014). Moreover, the continued
59 loss of seagrass ecosystems raises concerns that vast amounts of previously sequestered carbon could be
60 released back in the atmosphere, converting seagrasses from carbon sinks to carbon sources and
61 intensifying global climate change (Macreadie et al., 2013). The ongoing decline could potentially release
62 up to 299 Tg of carbon annually, contributing roughly 10% of CO₂ emissions associated with
63 anthropogenic land-use changes (Fourqurean et al., 2012).

64

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

76
77 Although there is increasing consensus on the potential of “Blue Carbon” storage in seagrass meadows
78 as a climate change mitigation strategy, the biogeochemical cycling within these ecosystems is complex.
79 Several processes, including ecosystem calcification, anaerobic metabolism, and bioturbation, can
80 counteract net organic carbon (OC) sequestration (Van Dam et al., 2021). These processes regulate local
81 DIC and total alkalinity (TA) budgets, adding complexity to accurately quantifying carbon sequestration
82 (Kindeberg et al., 2024). Overlooking these processes can result in significant overestimates of local
83 carbon sequestration rates and misinterpretations of the role seagrass meadows play in mitigating climate
84 change, potentially leading to inaccurate assessments of their carbon sink capacity (Johansen et al., 2023;
85 Chen et al., 2024; Fan et al., 2024).

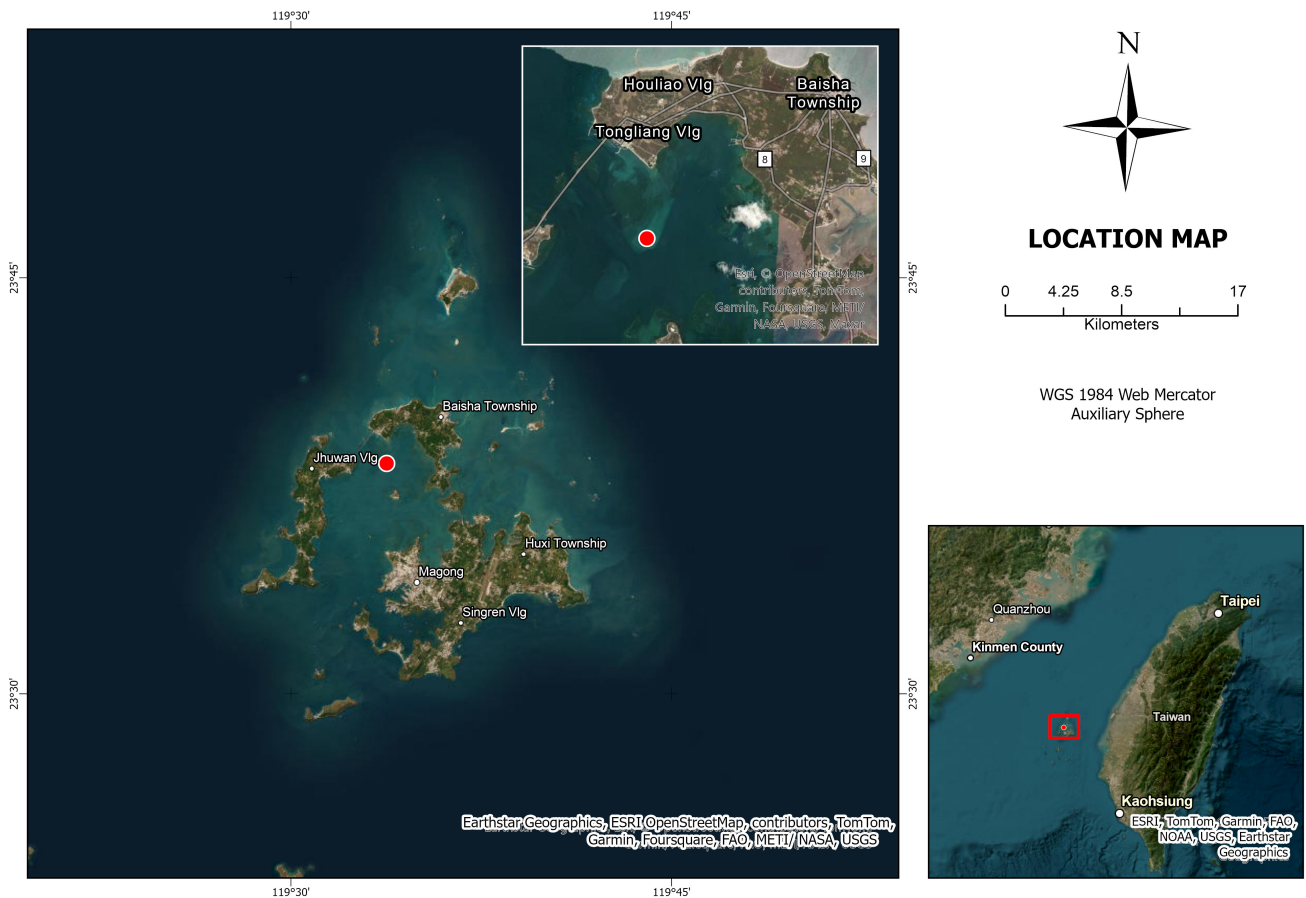
86
87 Several methodologies were developed to quantify benthic metabolism, which is a crucial component of
88 biogeochemical cycling, including photosynthesis-irradiance curve (Kraemer and Alberte, 1993), the
89 open water O₂ mass balance approach (Odum, 1956; Chou et al., 2023), and aquatic eddy covariance
90 (Berg et al., 2022; Juska and Berg, 2022). While these methods provide important data, they might
91 overlook the complexities of bioturbation, remineralization, and carbonate dynamics (Olivé et al., 2016;
92 Ward et al., 2022; Juska and Berg, 2022). In this study, we aim to address these knowledge gaps by

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

96 **2 Materials and Methods**

97 **2.1 Study site**

98 The Penghu Islands, located in the southern part of Taiwan Strait (Fig. 1), host a range of seagrass species.
99 Notably, four species have been reported: *Halophila ovalis*, *Halodule pinifolia*, *Halodule uninervis*, and
100 *Zostera japonica* (Yang et al., 2002). The sampling location (23° 38' 18.38" N and 119° 33' 46.48" E) is
101 a restoration meadow dominated by *H. uninervis* and *H. ovalis*. This restoration site encompasses
102 approximately 3 hectares (Allen Coral Atlas, 2020), with seagrass percent cover varying from 20% to
103 90%. These seagrasses are subtidal, with water depths ranging from 1.7 meters to 4.4 meters. The
104 substrate in this area is composed of carbonate sand. The area supports a diverse community of bivalves
105 (e.g., *Pinna* sp.), gastropods, echinoderms, and various fish species, all of which were observed during
106 the sampling.



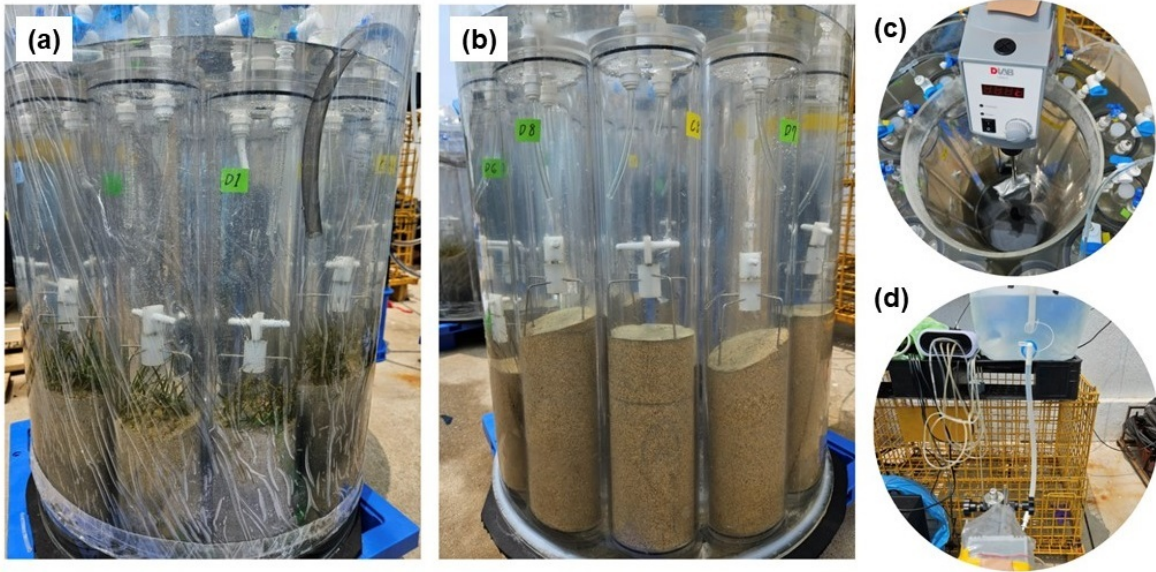
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108 **Figure 1: Location map of sampling stations in restored seagrass in Penghu Island, Taiwan (Map**
 109 **created in ArcGIS Pro. Source: Earthstar Geographics, ESRI OpenStreetMap, Contributors,**
 110 **TomTom, Garmin, Foursquare, FAO, METI/NASA,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

111 2.2 Ex situ core incubation system

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

118 core was sealed with a plexiglass lid containing two ports, one for probe insertion (Eyre & Ferguson,
119 2005). This method was used to quantify seagrass metabolism, particularly in subtidal systems where in
120 situ measurements are often logistically challenging. While ex situ conditions may differ from natural
121 underwater environments, we carefully designed our setup to closely replicate field conditions, including
122 natural light exposure and ambient temperature, to ensure ecological relevance.



123

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

128 2.3 Sediment core collection and pre-incubation

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

135

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

143 **2.4 Sample collection and analysis**

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

150

151 After measurements, three 150 ml seawater samples were collected separately from the SG and BS cores
152 using a syringe for DIC and pH analysis. The water samples were processed with 60 µL HgCl₂ solution
153 to stop any biological activity. DIC analysis was performed using a non-dispersive infrared method with
154 a DIC analyzer (AS-C3, Apollo SciTech Inc.), following the approach of Dickson et al. (2007) and our
155 past studies (Chou et al., 2018; 2021; Fan et al., 2024). For each DIC run, we used certified reference
156 material (Batch no. 206) sourced from A. G. Dickson at Scripps Institution of Oceanography to check for
157 drift and systematic bias. pH values were measured spectrophotometrically in total scale at 25 °C
158 following Clayton and Byrne (1993). Data from DIC and pH, along with actual temperature and salinity,
159 were used to calculate the TA, partial pressure of CO₂ (*p*CO₂), and aragonite saturation state (Ω_{Ar}) using
160 the Excel macro CO2SYS version 2.1 (Pelletier et al. 2011). The dissociation constants for carbonic acid

161 applied in these calculations were obtained from Mehrbach et al. (1973) and subsequently refined by
162 Dickson and Millero (1987).

163 **2.5 Benthic flux rate calculations**

164 Areal rates of R, GPP, NPP, and NEM were calculated based on changes in DO concentrations, following
165 equation 1 (Eyre et al. 2011). Respiration rates were determined from concentration data collected during
166 the initial dark period (midnight to dawn) (eq. 2). NPP was calculated based on light O₂ flux
167 measurements from dawn to noon (eq. 3). We implemented a 6-hour dark incubation period to ensure
168 oxygen concentrations remained above 80% (Eyre et al., 2002) and a 6-hour light incubation period to
169 prevent oxygen from reaching supersaturated levels (Olivé et al., 2016). Hourly GPP rates were computed
170 as the difference between R and NPP rates (eq. 4). NEM was calculated using equation 5. Positive values
171 indicate autotrophic, while negative values represent heterotrophic.

$$172 \quad F = [(C_{t1} - C_{t0}) \times V/A]/T \quad (\text{eq. 1})$$

173 Where F = flux rate ($\mu\text{mol m}^{-2} \text{h}^{-1}$), C_{t0} and C_{t1} = concentration in the overlying water at the start and end
174 of the time period ($\mu\text{mol l}^{-1}$), respectively, V = volume of overlying water in the core (l), A = surface area
175 in the sediment core (m²), and T = incubation period (h).

$$176 \quad R = \text{dark O}_2 \text{ flux (negative)} \quad (\text{eq. 2})$$

$$177 \quad \text{NPP} = \text{light O}_2 \text{ flux (positive)} \quad (\text{eq. 3})$$

$$178 \quad \text{GPP} = \text{NPP (positive)} - R \text{ (negative)} \quad (\text{eq. 4})$$

$$179 \quad \text{NEM} = (\text{GPP} \times 12) - (R \times 24 \text{ h} \times -1) \quad (\text{eq. 5})$$

180

181 NEC rates ($\text{mmol CaCO}_3 \text{ m}^{-2} \text{h}^{-1}$) were estimated from the change of total alkalinity, assuming these
182 changes are only due to CaCO₃ precipitation and dissolution (eq. 6) (Roth et al., 2019; Van Dam et al.,
183 2019):

$$184 \quad \text{NEC} = -0.5 \frac{\Delta n\text{TA}}{\Delta t} \times hp \quad (\text{eq. 6})$$

185 Here, $\Delta n\text{TA}$ = change in nTA ($n\text{TA} = \text{TA} \times \text{SSS}_{\text{average}}/\text{SSS}$) over the Δt (time), h = volume/area, and p =
186 water density. The -0.5 scalar factor was applied to account for the stoichiometric relationship, where 2

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

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

196 **2.5 Statistical analysis**

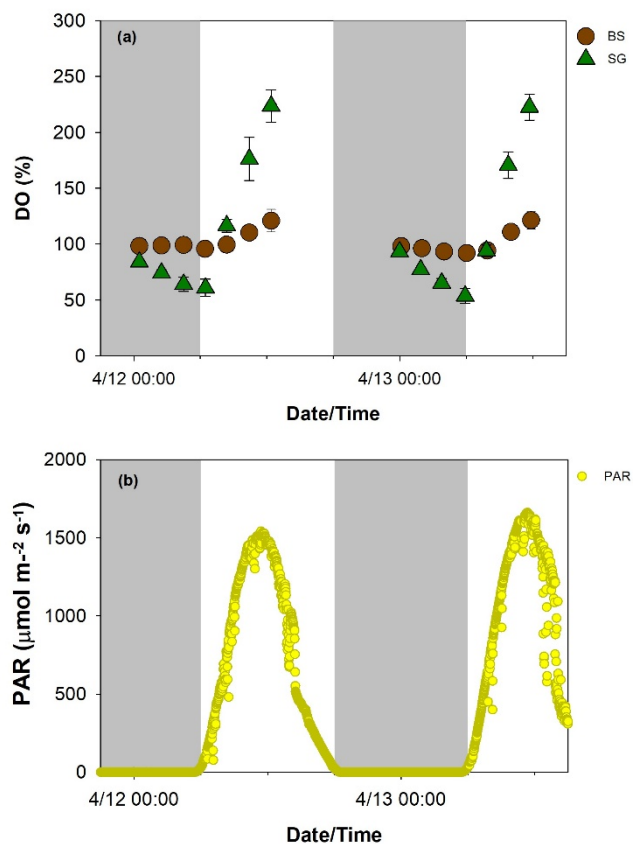
197 Independent sample T-tests were applied to compare metabolic rates (R, NPP, GPP, NEM, NEC) between
198 SG and BS using SPSS v. 17. Data were subjected to a normality test before performing the analysis.
199 Least-squares linear regression was employed to assess the correlation between changes in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

202 **3 Results**

203 **3.1 Water quality and carbonate chemistry**

204 Diurnal patterns of water quality and carbonate parameters for SG and BS during the two-day ex situ core
205 incubation are illustrated in Figs. 3 and 4, respectively. The temperature in both treatments ranged from
206 22 to 29 °C, while salinity levels spanned from 35 to 36. These values were similar to in situ measurements
207 obtained from the seagrass beds using a CTD profiler. During the daytime (6:00 AM to 12:30 PM), PAR
208 levels ranged from 26 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to a peak of 1662 $\mu\text{mol m}^{-2} \text{s}^{-1}$, with the highest intensities observed
209 at midday. The average PAR measured 953 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on the first day of incubation, increasing slightly
210 to 1026 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on the second day. DO saturation levels were more variable in SG than BS, with

211 values ranging from 54% to 224% and 92% to 123%, respectively. DO saturation levels in both treatments
212 followed a diel pattern, with lower nighttime and higher daytime values.
213
214 Both $nDIC$ ($nDIC = DIC \times SSS_{\text{average}}/SSS$) and pH_T displayed greater diurnal fluctuations at SG compared
215 to the BS. At SG, $nDIC$ ranged from 1660 to 2118 $\mu\text{mol kg}^{-1}$ (mean $\pm$ SD: $1963 \pm 153 \mu\text{mol kg}^{-1}$), and
216 followed a diel pattern. pH_T ranged from 7.81 to 8.37 at SG (mean $\pm$ SD: 7.99 ± 0.2), following the
217 opposite trend to $nDIC$, with values decreasing at night and increasing during the day. At the BS site,
218 these parameters were less variable, with $nDIC$ values ranging from 1948 to 2029 $\mu\text{mol kg}^{-1}$ and pH_T
219 from 7.84 to 7.99, with mean values of $1993 \pm 27 \mu\text{mol kg}^{-1}$ and 7.93 ± 0.1 , respectively. Similarly, the
220 calculated nTA was also more fluctuating in SG than BS, with mean values of $2243 \pm 24 \mu\text{mol kg}^{-1}$ and
221 $2230 \pm 24 \mu\text{mol kg}^{-1}$, respectively. The calculated pCO_2 displayed a broader range at SG (142 to 762
222 μatm ; mean $\pm$ SD: 510 ± 231) compared to BS (450 to 699 μatm ; mean $\pm$ SD: 524 ± 82), suggesting a
223 more dynamic carbon cycling potentially driven by seagrass metabolic activity. The mean Ω_{Ar} was higher
224 in SG (3.14 ± 1) compared to BS (2.72 ± 0.4), indicating more favorable conditions for calcification at
225 the seagrass site. Mann–Whitney test on carbonate chemistry revealed no significant distinction between
226 SG and BS ($pH_T p = 0.713$; $nDIC p = 0.419$; $nTA p = 0.679$; $\Omega_{Ar} p = 0.511$).



227
 228 **Figure 3: Diurnal pattern of dissolved oxygen (DO, a) in replanted seagrass (SG, green triangle)**
 229 **and bare sediment (BS, brown circle) (n=9, mean $\pm$ SD), and photosynthetically active radiation**
 230 **(PAR, b) during the two-day (April 12-13, 2024) incubation.**

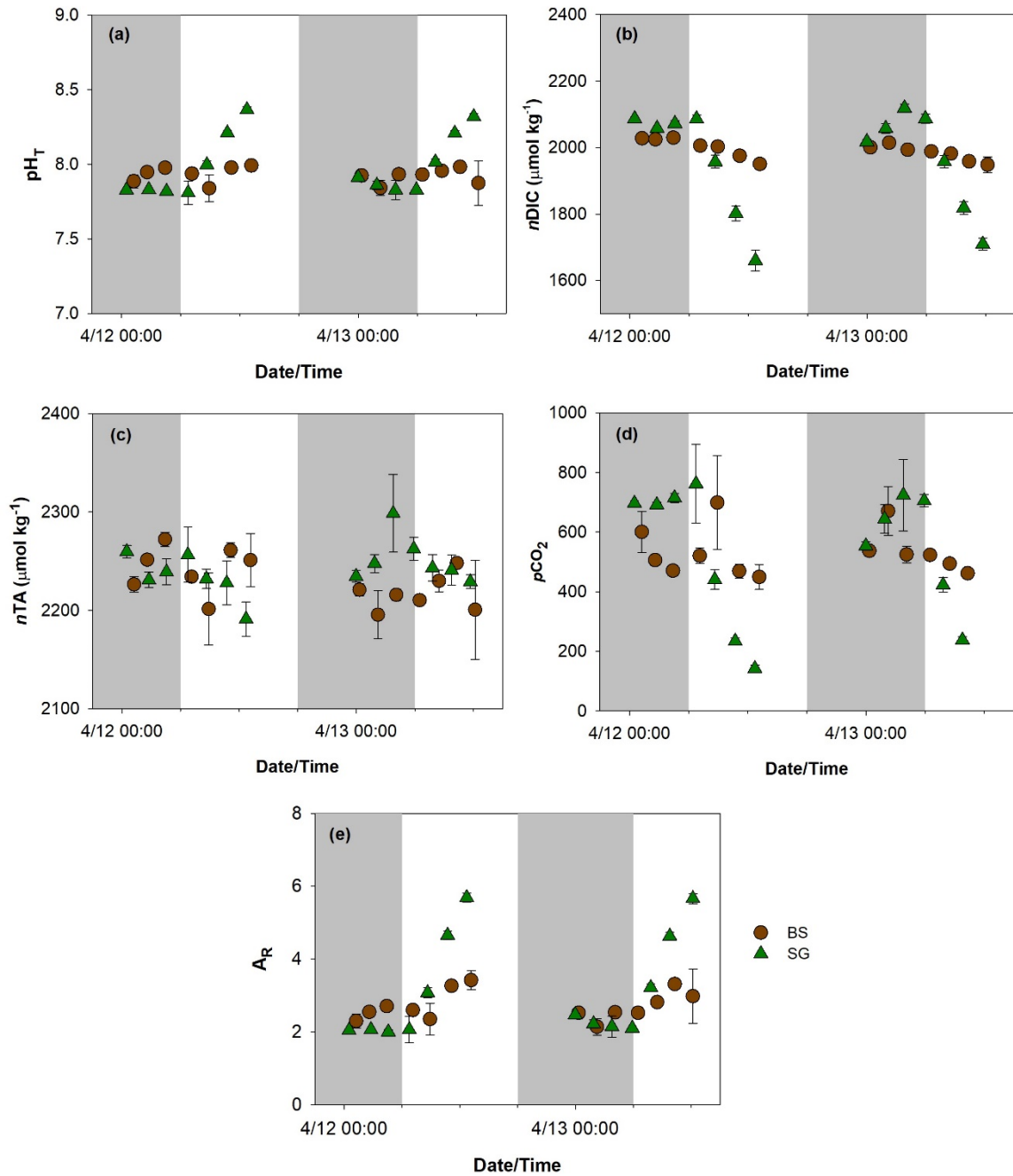


Figure 4: Total scale pH (pH_T , a), normalized dissolved inorganic carbon ($nDIC$, b), normalized total alkalinity (nTA , c), partial pressure of carbon dioxide (pCO_2 , d), and aragonite saturation state (Ω_{AR} , e) in replanted seagrass (SG, green triangle) and bare sediment (BS, brown circle) during the two-day (April 12-13, 2024) incubation. $n=3$, mean $\pm$ SD.

236 3.2 Respiration, gross primary production, and net ecosystem metabolism

237 Figure 5 illustrates the comparison of metabolic rates (mean $\pm$ SD) between SG and BS. The mean
238 respiration rates in SG (-4.3 ± 1.5 mmol O₂ m⁻² h⁻¹) were significantly higher than in BS (-0.6 ± 0.4 mmol
239 O₂ m⁻² h⁻¹), by approximately 8-fold difference ($p < 0.01$). The mean GPP in SG was 26.0 ± 3.4 mmol O₂
240 m⁻² h⁻¹, which is 35-fold higher than in BS (0.7 ± 1.3 mmol O₂ m⁻² h⁻¹) ($p < 0.01$). GPP was always higher
241 than R in both systems, with mean GPP/R ratios of 3.4 and 1.9 in SG and BS, respectively. For NEM,
242 both systems displayed positive values, indicating net autotrophy, with SG being 10-fold higher ($208.2 \pm$
243 22.2 mmol O₂ m⁻² d⁻¹) compared to BS (20.1 ± 9.9 mmol O₂ m⁻² d⁻¹) ($p < 0.01$). Both R and GPP in SG
244 and BS increased on the second day of incubation [SG (R: -3.1 vs -5.6 mmol O₂ m⁻² h⁻¹; GPP: 23.3 vs
245 24.7 mmol O₂ m⁻² h⁻¹); BS (R: -0.4 vs -0.81 mmol O₂ m⁻² h⁻¹; GPP: 2.7 vs 3.1 mmol O₂ m⁻² h⁻¹)], while
246 NEM in SG (218.04 vs 198.4 mmol O₂ m⁻² d⁻¹) and BS (22.3 vs 17.8 mmol O₂ m⁻² d⁻¹) showed a slight
247 decrease. However, these changes were not statistically significant.

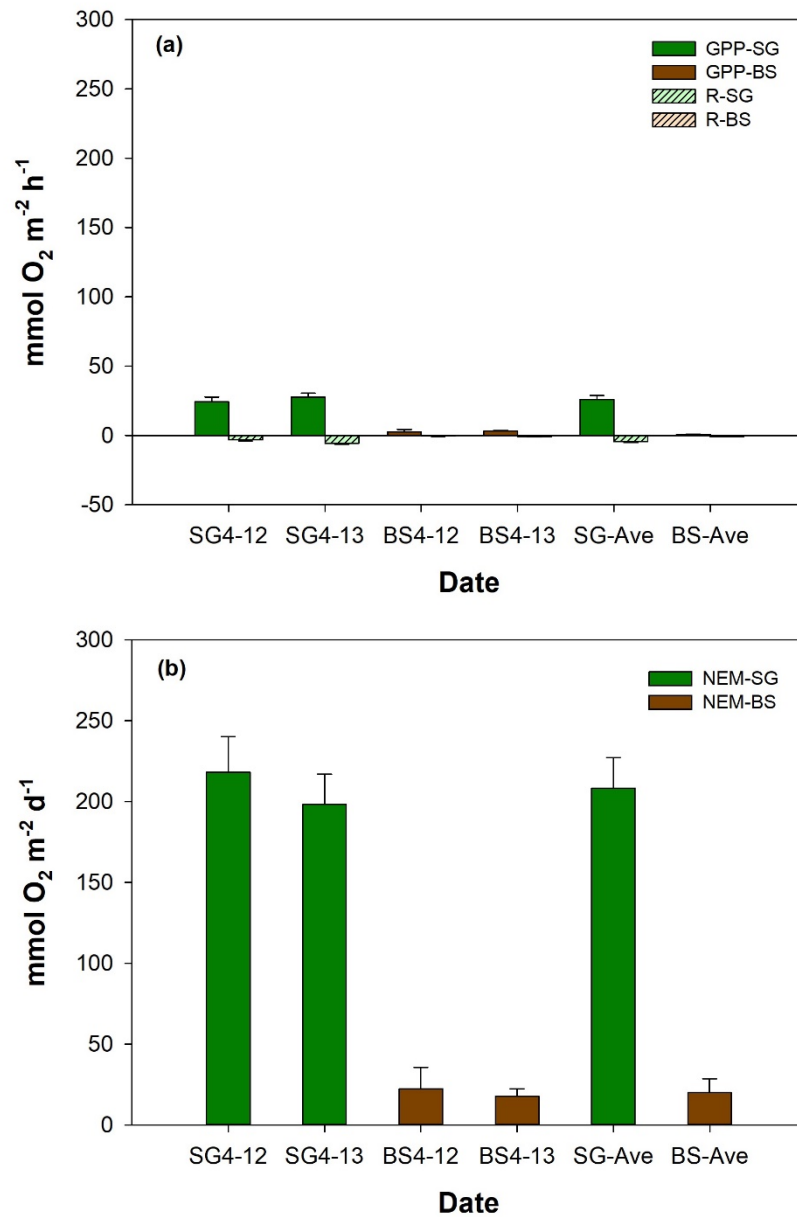


Figure 5: Mean ($\pm$ SD, standard deviation) values of (a) metabolic rates such as respiration (R), gross primary productivity (GPP), and (b) net ecosystem metabolism (NEM,) of restored seagrass (SG, green bars) and bare sediment (BS, brown bars) in Penghu during the two-day (April 12-13, 2024) incubation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

254 3.2 Calcium carbonate precipitation, dissolution, and net ecosystem calcification

255 The NEC values (mean $\pm$ SD) over a diel cycle for SG and BS demonstrated differences in their overall
256 carbonate dynamics (Fig. 6). Over the two-day incubation period, SG exhibited a net calcifying system
257 with a mean positive daily NEC means (10.9 ± 15.7 mmol CaCO_3 m^{-2} d^{-1}), driven by daytime calcification
258 (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,
259 BS supported a net-dissolving system with mean daily NEC (-2.3 ± 18.8 mmol CaCO_3 m^{-2} d^{-1}). Mean
260 daytime calcification and nighttime dissolution were 0.1 ± 1.6 mmol CaCO_3 m^{-2} h^{-1} and -0.2 ± 0.6 mmol
261 CaCO_3 m^{-2} h^{-1} , respectively. Both systems followed a general diurnal pattern, with positive NEC during
262 the day (calcifying) and negative at night (dissolving).

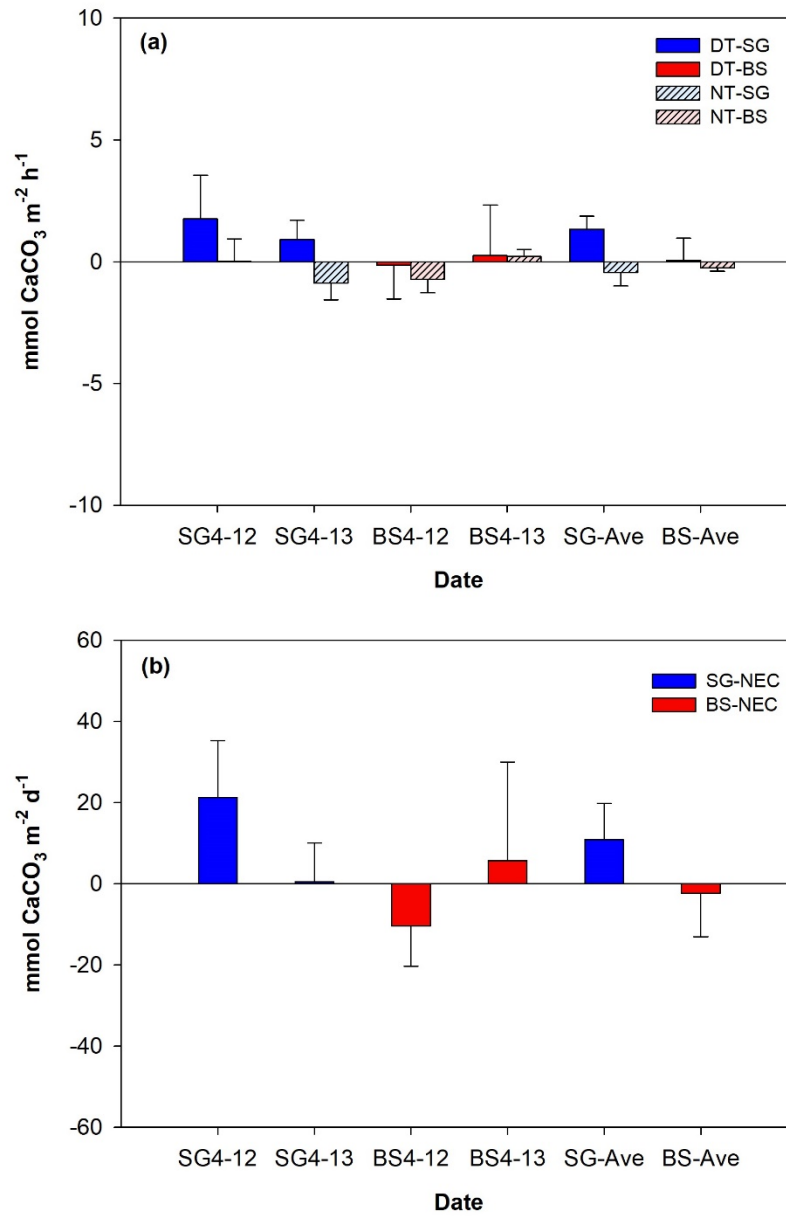


Figure 6: Mean ($\pm$ SD, standard deviation) values of daytime (DT) and nighttime (NT) calcification (a), and net ecosystems calcification (NEC, b) of restored seagrass (SG, blue bars) and bare sediment (BS, red bars) in Penghu during the two-day (April 12-13, 2024) incubation (n=3).

267 4 Discussion

268 Seagrass meadows are widely recognized as an important blue carbon ecosystem with substantial
269 potential to mitigate anthropogenic CO₂ emissions. Although research on seagrass ecosystems has grown
270 in recent years, significant gaps remain in understanding their carbon dynamics. In particular, the balance
271 of organic and inorganic carbon processes within these systems is not fully understood. Meanwhile, global
272 seagrass coverage continues to decline, which has increased the urgency of restoration efforts (Waycott
273 et al. 2009). Restoring seagrass meadows to enhance carbon sequestration has become increasingly
274 important. Currently, most studies on restored seagrass meadows focus primarily on the burial of
275 particulate organic carbon (Greiner et al. 2013), with far fewer exploring both organic metabolism and
276 carbonate cycling in restored seagrass meadows. Here, we present the first dataset on carbon uptake
277 through metabolic rates and calcification measurements in restored seagrass meadows within tropical
278 regions.

279 4.1 Restoration of seagrass enhances metabolic rates

280 The metabolic rates estimated in present study were comparable to those recorded in other seagrass
281 meadows (Table 1). Our GPP in SG was 24% and 37% higher than the tropical and global averages,
282 respectively, but 38% lower than Dongsha Island, Taiwan (Chou et al., 2023). It is also comparable to
283 measurements reported for *H. uninervis* in Tropical Australia (Table 1). Conversely, the R values
284 estimated in this study were roughly half lower than the tropical and global averages (Duarte et al., 2010).
285 Our NEM (214 mmol O₂ m⁻² d⁻¹) is within the range of previous estimates for tropical seagrass meadows
286 (-477.28 to 484.20 mmol O₂ m⁻² d⁻¹) and global estimates (-477.28 to 531.63 mmol O₂ m⁻² d⁻¹). In addition
287 to these global comparisons, our study reveals a clear distinction in metabolic rates (e.g. GPP, R, NEM)
288 between SG and BS. The GPP and R in restored seagrass meadows were 35 and 7 times greater than in
289 BS. The relatively higher metabolic rates in seagrass meadows compared to bare sediments have also
290 been observed in other studies (Table 1). For instance, a two-year-old restored *Halodule wrightii* meadow
291 demonstrated a 13-fold increase in NEM relative to bare sediment (Egea et al., 2023). Similarly,
292 *Posidonia oceanica* exhibited a notable 70-fold increase in metabolic rates compared to bare sediment
293 (Barron et al., 2006). Furthermore, *Zostera marina* exhibits net autotrophy while bare sediments are net

294 heterotrophy (Attard et al., 2019; Chen et al., 2019). Such patterns highlight the fundamental ecological
 295 functions restored seagrass meadows play relative to unvegetated/bare sediments. The increase in GPP
 296 reflects the enhanced carbon fixation capacity of seagrass meadows, while the elevated R indicates active
 297 organic matter decomposition and microbial respiration (Duarte and Krause-Jensen, 2017). According to
 298 Duarte et al. (2010), seagrass meadows generally act as autotrophic ($NEM > 0$) CO_2 sinks when GPP
 299 exceeds $186 \text{ mmol } O_2 \text{ m}^{-2} \text{ d}^{-1}$, and shift to heterotrophy ($NEM < 0$) when GPP falls below this threshold.
 300 Based on this threshold, our mean GPP for restored seagrass exceeded the value for autotrophy, resulting
 301 in a positive NEM which is consistent with their global assessment. The NEM observed in SG was 10
 302 times higher than in BS, suggesting that SG sequesters significantly more carbon than BS. These findings
 303 highlight that seagrass restoration significantly boosts metabolic rates and enhances carbon cycling.
 304 Given the increasing loss of global seagrass cover, restoration not only boosts ecosystem productivity but
 305 also strengthens the ability of coastal systems to remove carbon, thereby contributing to climate change
 306 mitigation efforts.
 307

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

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

Finland	Aquatic eddy covariance	Bare sediment	1.60	0.82	-0.14	Attard et al., 2019
		<i>Z. marina</i>	3.74	1.71	4.17	
Australia	Ex situ benthic	Bare sediment	2.28	1.26	-2.74	Chen et al., 2019
		<i>Zostera</i> sp.	6.94	2.74	7.12	
		<i>Halophila</i> sp.	2.05	1.60	-13.70	
Tropical Australia	Combined methods	<i>H. uninervis</i>	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
		<i>P. oceanica</i>	7.72	3.18	16.44	

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

312

313 Key drivers of elevated metabolic rates in tropical meadows include greater PAR availability,
314 aboveground biomass, and higher temperatures (Ganguly et al., 2017; Ward et al., 2022). Many tropical
315 species grow near their optimal photosynthetic and physiological conditions (Lee et al., 2007; Koch et al.,
316 2012), efficiently capturing light in shallow, clear waters, which contributes to higher NEP (Ralph et al.,
317 2007). In our study, DO variation corresponds to light intensity (Figs. 3 and 7), suggesting that the
318 elevated GPP observed in seagrass meadows could be driven by higher light intensity. This is likely due
319 to the relatively lower canopy cover of *H. uninervis* and density in SG, which reduces shading within the
320 seagrass. As a result, more light penetrates to the leaves, increasing their photosynthetic surface area and
321 contributing to NEM (Ralph et al., 2007). In contrast, lower respiration rates in the SG area were likely
322 due to the sediment characteristics and organic matter quality in this habitat. The seagrass beds are situated
323 in carbonate-rich sediments, which typically contain less organic matter than siliciclastic or muddy
324 sediments (Belshe et al., 2018; Kindeberg et al., 2018). This limits the availability of substrates for
325 microbial decomposition. Moreover, the organic matter derived from seagrass detritus is generally more
326 refractory and less labile, further reducing its accessibility for microbial breakdown and thereby
327 suppressing heterotrophic respiration (Ren et al., 2024). Although seagrasses are capable of transporting

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.

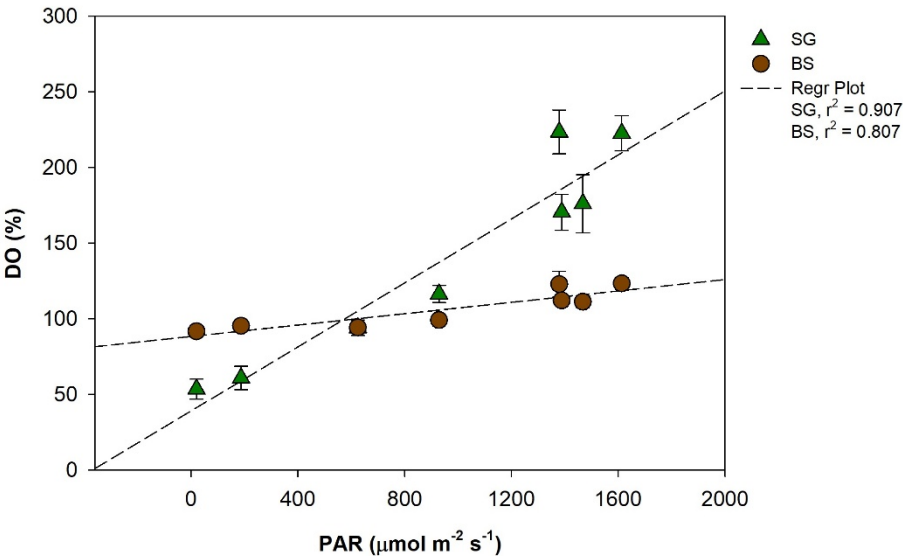


Figure 7: Regression plot between photosynthetically active radiation (PAR, $\mu\text{mol m}^{-2} \text{s}^{-1}$) vs dissolved oxygen (DO, %) in restored seagrass (SG, green triangle) and bare sediment (BS, brown circle). Error bars represent standard deviation (SD).

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

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

351 **4.2 Calcification dynamics in restored seagrass**

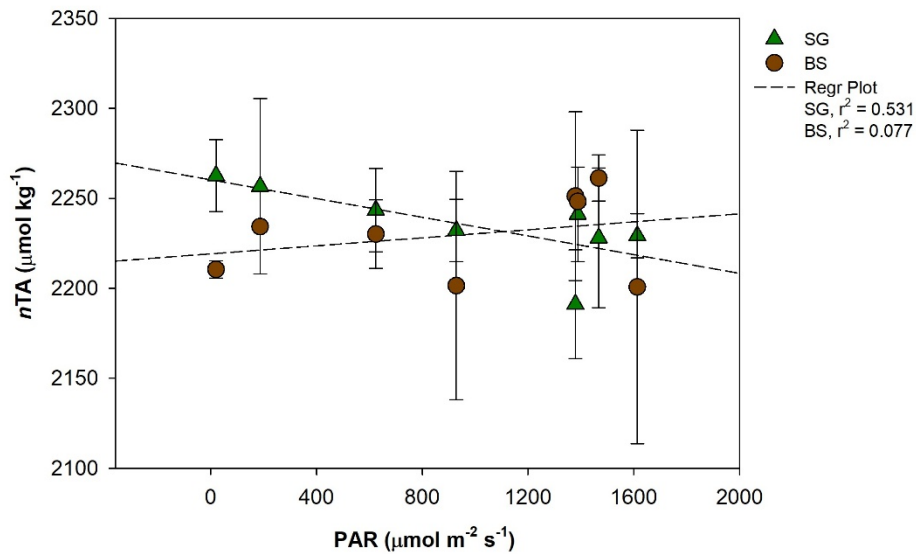
352 Our results show that restored seagrass meadows exhibit significantly higher CaCO_3 cycling — both
353 formation and dissolution — compared to bare sediments. This corroborates with prior studies, which
354 documented enhanced carbonate dynamics in vegetated habitats relative to unvegetated sediments.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

361

362 The variations of CaCO_3 production and dissolution in surface waters and sediment are related to the
363 carbon cycle through photosynthesis and respiration (Yates and Halley 2006). During daylight hours,
364 photosynthesis raises pH and reduces CO_2 levels in the water, creating favorable conditions for calcium
365 carbonate precipitation—a process referred to as light-enhanced calcification (Schneider et al., 2009).
366 We found a significant positive correlation between PAR and $n\text{TA}$ changes ($r^2 = 0.52$, $p < 0.05$), suggesting
367 that increased light availability may enhance calcification by photoautotrophs in restored seagrass areas
368 during the day (Fig. 8). Additionally, our data showed a significant negative correlation between $n\text{TA}$
369 flux and NEM ($r^2 = 0.54$, $p < 0.01$), indicating that higher photosynthetic activity (positive NEM) promotes
370 calcification by consuming TA, while lower NEM or net heterotrophy contributes to TA production,
371 likely through carbonate dissolution or anaerobic decomposition (Fig. 9). Similar relationships between
372 photosynthesis and calcification have been reported in marine calcifiers (Mallon et al., 2022), and the
373 influence of epiphytic organisms in promoting calcification during active photosynthesis has been

374 highlighted in seagrass meadows such as *P. oceanica* (Barrón et al., 2006). At night, carbonate dissolution
 375 predominates as aerobic respiration produces CO₂ and carbonic acid in sediment porewater (Eyre et al.,
 376 2014), lowering carbonate saturation and driving mineral dissolution (Burdige and Zimmerman, 2002;
 377 Burdige et al., 2008; Chou et al., 2021; Fan et al., 2024). The degree of dissolution is directly link to the
 378 rate of organic matter decomposition, which depends on the quantity of organic matter, its reactivity, and
 379 oxygen availability (Anderson et al., 2005; Morse et al., 2006). High shoot density and root biomass in
 380 restored seagrass meadows enhance organic matter supply and decomposition in sediment, further driving
 381 nighttime dissolution (Holmer et al., 2013).

382



383
 384 **Figure 8: Regression plot between photosynthetically active radiation (PAR, μmol m⁻² s⁻¹) vs**
 385 **normalized total alkalinity (nTA, μmol kg⁻¹) in restored seagrass (SG, green triangle) and bare**
 386 **sediment (BS, brown circle). Error bars represent standard deviation (SD).**

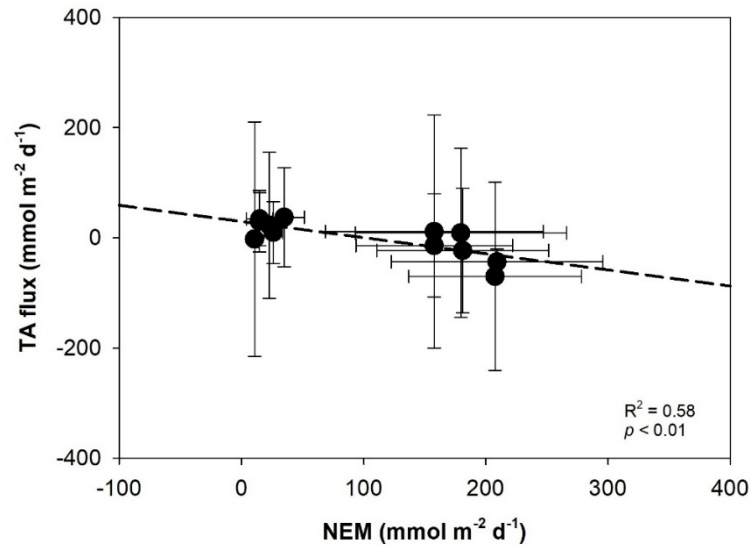


Figure 9: Linear regression showing the relationship between total alkalinity (TA, mmol m⁻² d⁻¹) flux and net ecosystem metabolism (NEM, mmol m⁻² d⁻¹) in restored seagrass meadows and bare sediment. Error bars represent standard deviation (SD).

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 CaCO₃ m⁻² yr⁻¹ (8.8 mmol CaCO₃ m⁻² d⁻¹) and 51 g CaCO₃ m⁻² yr⁻¹ (1.40 mmol CaCO₃ 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 CaCO₃ 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 CaCO₃ 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 CaCO₃ production, with tropical seagrass meadows typically supporting higher carbonate loads than temperate ones. Reported production rates

span from 180 g CaCO₃ m⁻² yr⁻¹ in Jamaica (Land, 1970) to 2800 g CaCO₃ m⁻² yr⁻¹ in Barbados (Patriquin, 1972), underscoring regional variability in seagrass-associated calcification. Moreover, fluctuations in CO₃²⁻ concentrations are crucial in regulating the capacity of calcifying organisms to form CaCO₃. 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 CO₂, 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 CO₂ 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.

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 CO₂ 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 CO₂ source or sink for each system. In the SG system, which is net calcifying, Φ indicates a CO₂ source, with 0.61 moles of CO₂ released into the seawater for each mole of CaCO₃ precipitated. In contrast, the BS system, which is net dissolving, Φ represents a CO₂ sink, with 0.65 moles of CO₂ absorbed for each mole of CaCO₃ dissolved. These values are comparable to previous findings, which reported a CO₂ flux-to-CaCO₃ 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 CO₂ m² d⁻¹ in SG and 20 mmol CO₂ m² d⁻¹ BS. For NEC, the carbon release in SG was 6.6 CO₂ m² d⁻¹, while for BS, an additional CO₂ uptake was -1.5 mmol CO₂ m² d⁻¹. Consequently, the net carbon uptake is 202 and 22 mmol CO₂ m² 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

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

434

435 **4.4 Limitations of ex situ benthic incubation and future research**

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

458 **5 Conclusion**

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

472 **Author contribution**

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

477 **Competing interest**

478 The authors declare that they have no conflict of interest.

479 **Data availability**

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

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