

A B S T R A C T

Small-hold aquaculture ponds are widespread in China, but their carbon greenhouse gas emissions are poorly quantified. In this study, we used a carbon budget approach to assess the climate footprint of three earthen aquaculture ponds in southeastern China with the whiteleg shrimp (*Litopenaeus vannamei*) during the farming period. The main carbon inputs to the ponds were planktonic primary production (58.5–61.8%), followed by commercial feeds (31.9-35.3%), while the major carbon outputs occurred through planktonic respiration (44.0–53.6%) and sedimentation 31 (18.0–21.7%). Water-to-air emissions of carbon greenhouse gases $(CO₂$ and CH₄) represented only a small fraction of the carbon flow (0.8–1.6%), with a combined CO₂-equivalent emission of 528.4±193.3 mg CO₂-eq m⁻² h⁻¹ based on GWP₂₀. We also observed significant spatio-temporal variation in carbon greenhouse gases among the three ponds, which could be attributed to the variation in Chl-*a* and carbon substrate supply. Nevertheless, the magnitude of CH4 emission from these ponds was still higher than some other agro-ecosystems. Moreover, we found that only 21% of the excess organic carbon was converted to shrimp biomass, while another 20% ended up in the sediment. Our findings suggested that lowering the feed conversion ratio and removing the bottom sediments regularly could help improve production efficiency, reduce the excessive accumulation of carbon-rich detritus and minimize the climatic warming impacts of aquaculture production.

Keywords: Aquaculture ponds; Carbon budget; Carbon dioxide; Methane; Global warming potential

List of abbreviations:

*W***S:** Wind Speed

1. Introduction

Since the Industrial Revolution, the atmospheric concentrations of the two main 60 greenhouse gases (GHGs), carbon dioxide $(CO₂)$ and methane (CH₄), have increased respectively by about 44% and 156% since 1750, reaching 419 ppm and 1.909 ppm,

respectively, in 2022 (NOAA, 2022). Aquatic habitats are important sources of global CO2 and CH4 emissions(Bastviken et al., 2011; Li et al., 2018; Tranvik et al., 2009; Yang et al., 2011); therefore, understanding the carbon greenhouse gas dynamics in these habitats will help mitigate global warming and the related impact on ecosystem (Yang et al., 2020).

Increasing global food demand has led to the rapid expansion of aquaculture world-wide, especially small-hold aquaculture ponds (FAO, 2018; Ren et al., 2019), raising concerns about their environmental impacts including GHG emissions (Datta et al., 2009; Frei et al., 2007; Yuan et al., 2019). Despite their small size, small-hold 71 aquaculture ponds can have high water-to-air $CO₂$ and $CH₄$ emissions, owing to their high productivities and shallow water depths (MacLeod et al., 2020; Yuan et al., 73 2019). Although there have been some efforts to characterize $CO₂$ and CH₄ fluxes across the water-air interface and their driving variables in aquaculture ponds (e.g., Bhattacharyya et al., 2013; Chanda et al., 2019; Soares and Henry-Silva, 2019), relevant data are still scarce for China, where small-hold aquaculture ponds are widespread but poorly researched (Hu et al., 2020, 2016; Ma et al., 2018; Yuan et al., 2019, 2021; Zhang et al., 2020a). Pond drainage, a common practice done in many small-hold aquaculture ponds, may divert the carbon downstream. On the other hand, carbon sequestration in the sediment may offset the 'climate footprint' of aquaculture ponds (Boyd et al., 2010). Proper assessment of the carbon greenhouse gas emissions and global warming potential of aquaculture ponds therefore requires accounting for both carbon inputs and outputs. Yet, such a mass balance approach is rarely used in aquaculture pond studies (Zhang et al., 2020b).

In the current study, we determined the different carbon input and output components of three earthen aquaculture ponds with *Litopenaeus vannamei* within a subtropical estuary in southeastern China. The research main objectives to: (1) assess 88 the carbon budgets of the earthen shrimp ponds, (2) quantify the contribution of $CO₂$ and CH4 emissions to the total carbon output, and (3) evaluate the role of aquaculture 90 ponds in driving global warming. We hypothesized that $CO₂$ and $CH₄$ emissions represent a major carbon loss, and the global warming effect of the shrimp ponds is on par with other food production systems. Based on the findings, we made recommendations to improve the production efficiency and minimize the climate footprint of shrimp aquaculture.

2. Materials and methods

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97 2.1. Study area
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The research was conducted in the Shanyutan Wetland (26°00′36″–26°03′42″ N, 119°34′12″–119°40′40″ E) of the Min River Estuary (MRE), southeastern China (Figure 1). It is the largest tidal wetland in the MRE, covering an area of approximately 3,120 ha. The MRE is influenced by the East Asian monsoon, with an

102 annual average precipitation of 1,350 mm and air temperature of 19.6 °C (Tong et al., 103 2010). The semidiurnal tidal range is 2.5–6.0 m and the average water salinity is 4.2 \pm 2.5 ‰. Aquaculture shrimp ponds, common within the Shanyutan Wetland and covering an area of ca. 234 ha (Yang et al., 2017a), were constructed by clearing away the marsh vegetation and re-profiling the bunds into slopes.

2.2. Aquaculture system and management practices

Field measurements were conducted in three coastal earthen aquaculture ponds that culture the whiteleg shrimps (*Litopenaeus vannamei*) (Figure 1). The selected ponds represent the typical management practices and physical setting of aquaculture in the MRE. They range in size of 1.25–1.40 ha and water depth of 1.3–1.6 m during 112 the farming period (Yang et al., 2021a). The farming period is usually between $5th$ 113 May and $8th$ November, producing a single crop. The ponds are drained and dried for the remainder of the year.

Prior to farming, the earthen ponds were filled with seawater drawn from the MRE. The seawater was first passed through a 2 mm mesh bag to exclude predators 117 and competitors. After seven days, trichloroisocyanuric acid $(\sim 25 \text{ kg pool}^{-1})$ was added to disinfect the pond water, followed by the addition of calcium oxide lime (0.5 119 t ha⁻¹) and calcium superphosphate fertilizer (1.5–2.0 kg per 1000 m³). Before shrimp 120 stocking, probiotics (200 mL ha^{-1}) were added, and water conditions (e.g., alkalinity or acidity, salinity, etc.) were checked to make sure they were in the correct range. 122 Each earthen pond was stocked with *L. vannamei* at a density of 215 post larvae m⁻². Commercial feed pellets were added daily during the farming period. Aeration was provided by aerators in the ponds. After harvesting, the pond water was discharged.

2.3. Carbon budgets of the shrimp ponds

The carbon budget was constructed by accounting for the different input and output components. Input equaled the sum of carbon input from the stocked shrimps, feeds, fertilizers, primary production of phytoplankton, inflow water and rainwater. Output equaled the sum of carbon loss through plankton respiration, sediment respiration, net carbon greenhouse gas emissions (CO2 and CH4), harvested shrimps, outflow water and sediment accumulation. Each of the aforementioned input and output terms was measured independently in this study, as explained below.

2.3.1. Input: Stocked shrimps and feeds

The initial stocking of shrimp biomass and daily feed amounts were recorded. Samples of the stocked shrimps and feeds were collected and oven-dried for 24 h at 136 60 °C (Dien et al., 2018), grounded and sieved through a 0.15 mm mesh screen, and their total carbon (TC) contents were analysed using a combustion analyzer (Elementar Vario MAX CN, Germany). The detection limit and relative standard 139 deviations were 4 μ g L⁻¹ and ≤1.0% for TC, respectively.

2.3.2. Input: Primary production of phytoplankton

During each sampling campaign (May-October; 2-3 times per month), phytoplankton primary production was determined by the light−dark bottle oxygen method (Diana et al., 1991; Zhang et al., 2016, 2020b). Water samples from the surface (20 cm) and bottom (ca. 5 cm above the sediment) layers was sampled at five stations in each pond to measure the initial dissolved oxygen (DO) concentration by the Winkler method (Diana et al., 1991; Chen et al., 2018). Two dark and two light bottles (200 mL) were filled with ambient waters and suspended in situ at the original depths, and the final DO concentrations in the bottles were determined after 24 h. Gross primary production (P_P) and plankton respiration $(R_P; \text{mg } O_2 L^{-1})$ were calculated from the changes in DO concentrations as follows (Zhang et al., 2016):

$$
P_{\rm P} = \rm{DO}_{L} - \rm{DO}_{L} \tag{Eq.1}
$$

$$
R_{\rm p} = \rm{DO}_{I} - \rm{DO}_{D} \tag{Eq.2}
$$

153 where DO_L (mg L^{-1}) and DO_D (mg L^{-1}) are the final DO concentrations in the light 154 and dark bottles, respectively; DO_I (mg $L⁻¹$) is the initial dissolved oxygen. 155 Measurements were converted to carbon using the conversion of 1 mg O_2 to 0.375 mg C (Guo et al., 2017; Winberg, 1980).

2.3.3. Input: Inflow water and rainwater

Samples of inflow water were collected from two inlets using an organic glass hydrophore at each pond, while rainwater was sampled by a rain gauge 5 times during the farming period. All the water samples were stored in a cold and dark container and transferred back to laboratory for further analysis within 4–6 h. Approximately 50 mL of the water sample was filtered through a pre-combusted 0.45 μm glass fibre filter to separate the particulate organic carbon (POC) and dissolved organic carbon (DOC) fractions. Both fractions were subsequently analyzed using a total organic carbon (TOC) analyzer (TOC–VCPH/CPN, Shimadzu, Japan). In addition, rainfall data from a local weather station were used to estimate the total precipitation entering each pond during the farming period.

168 *2.3.4. Output: Water-column and sediment respiration*

Respiration in the water column by both autotrophic and heterotrophic plankton was derived from the light-dark bottle incubations described earlier (Eq. 2) (Zhang et al., 2016, 2020b). Sediment respiration was measured with a sediment incubation chamber (30 cm length, 6 cm internal diameter; Yang et al. 2017b, 2019). Surface sediment (0-15 cm) was taken from five sites in each pond using a metal corer (diameter 6 cm). The sediment samples were sealed in vacuum inside the incubation chambers. Both sediment and pond water samples were transported to the laboratory within 4 hr, and then were allowed to equilibrate to the lab condition for 2 h (Zhang et 177 al., 2016). The incubation chamber was filled with pond water up to 15 cm above the surface of sediment, and then sealed with a Teflon stopper. The incubation was done in an incubator (QHZ-98A, Taicang, Jiangsu, China) at in-situ temperature for 4 h in darkness. Initial and final samples of the overlying water were taken from the chamber to determine DO by Winkler titration. Incubation chambers filled with pond water only (no sediment) was used as the control. Sediment oxygen demand (SOD, 183 mg O_2 m⁻² d⁻¹) was determined from the change in DO in the overlying water:

> (Eq.3) $SOD = \frac{(DO_{I} - DO_{F})}{T}$ $\mathrm{SC} \wedge \mathbf{I}$ IE I^{\sim} D \cup _F \prime \sim \prime ow $A_{\rm SC} \times T$ *V* $\overline{\mathsf{x}}$ $=\frac{(DO_{I}-DO_{F})\times}{(DO_{I}-DO_{F})}\times$

185 where DO_I and DO_F (mg $O₂$ L⁻¹) are the initial and final DO concentrations, 186 respectively; *V*_{OW} (L) is the volume of overlying water in the incubation chambers, 187 A_{SC} (m²) is the cross-sectional area of the sediment core, T_{IE} (h) is the duration of the 188 incubation experiment. SOD (mg O_2 m⁻² h⁻¹) was converted to carbon demand (mg C 189 $\text{m}^{-2} \text{ d}^{-1}$ (1 mg O₂ = 0.375 mg C) according to Winberg (1980).

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At the end of the farming period, the ponds were drained and the shrimps were harvested, weighed, and analyzed for TC as described above. Outflow water was collected from two outlets at each pond using an organic glass hydrophore. The water samples were analysed for POC and DOC using the same methods as described before (section 2.3.3).

Similar to previous studies (Pouil et al., 2019; Zhang et al., 2016, 2020c), carbon sedimentation was derived from sediment height accumulation over time and 198 sediment carbon content. Briefly, five 0.5 m^2 ceramic tiles were placed inside each of the ponds at the start of the farming period, and the height of the accumulated sediment (cm) on the tiles at the end of the farming period was measured by vernier caliper. Additionally, sediment samples (0–20 cm depth) were collected on a monthly basis from five sites in each pond using a metal corer (diameter 6 cm). In the laboratory, the sediment TC were determined using an Elementar combustion analyzer (Elementar Vario MAX CN, Germany). The accumulated sediment height and sediment TC content were then used to estimate the total amount of carbon sedimentation throughout the farming period (Flickinger et al., 2020).

2.3.6. Output: Carbon greenhouse gas emissions

208 The fluxes of $CO₂$ and CH₄ across the water-air interface (WAI) were determined using the opaque floating chamber method, the details of which can be found in Natchimuthu et al. (2016). Briefly, the floating chamber had a volume of 5.2 L 211 covering a surface area of 0.1 m². During each campaign, CO_2 and CH_4 flux measurements were made at five sites in each pond. At each site, gas samples were collected from the floating chamber air headspace at 0, 15, 30, and 45 min, and were then transferred into sample bags. Sampling was done between 9:00 and 11:00 local 215 time outside of the time when the aerators were running. $CO₂$ and $CH₄$ concentrations in the gas sampled were analysed within 24 h by a gas chromatograph equipped with 217 a flame ionization detector (GC-2010, Shimadzu, Kyoto, Japan). CH₄ fluxes determined by the floating chamber method represented the sum of ebullitive CH⁴ 219 fluxes and diffusive CH₄ fluxes (Wu et al., 2019; Zhu et al., 2016). Gas fluxes (CO₂ or 220 CH₄; mg C m⁻² h⁻¹) across the WAI were estimated as the rate of change in the mass of CO_2 (or CH₄) per unit surface area per unit time (Yuan et al., 2021; Zhu et al., 2016) as follows:

$$
F = \frac{dc}{dt} \bullet \frac{M_M}{V_M} \bullet \frac{P}{P_0} \bullet \frac{T}{T_0 + T} \bullet H
$$
 (Eq. 4)

224 where *F* is the fluxes of CO₂ or CH₄ (mg C m⁻² h⁻¹); dc/dt is the slope of the CO₂ (or CH₄) concentration (*c*, mmol mol⁻¹) curve variation over time (*t*, hour); M_M is the 226 molar mass of CO₂ or CH₄ (mg mol⁻¹); V_M is the gas molar volume (m³ mol⁻¹); P_0 and 227 *T*₀ is the atmospheric pressure (kPa) and absolute temperature (K), respectively, under 228 the standard condition; *P* and *T* is the air pressure (kPa) of the sampling pond and the 229 air temperature (K) during the measurement, respectively; *H* is the floating chamber 230 height (m) over the water surface.

231 2.4 . Total CO₂-equivalent (CO₂-eq) emission

232 Because different GHGs have different degrees of radiative forcing over different 233 time scales, to aid comparison and policy development, their respective warming 234 effects are often expressed as CO_2 -equivalent on a chosen time horizon by applying 235 the appropriate global warming potential values (Skytt et al., 2020). In the present 236 study, we multiplied the mass of CH₄ by a global warming potential (GWP₂₀) value of 237 84 to calculate its CO_2 equivalent $(CO_2$ -eq) on a 20-year time horizon (Fang et al., 238 2021 ; IPCC, 2014). This was then added to the mass of $CO₂$ emission to calculate the 239 total $CO₂$ -eq emission on a 20-year time horizon.

240 *2.5. Measurements of ancillary environmental variables*

241 Meteorological data such as wind speed (W_S) , air temperature (T_A) , and air 242 pressure (A_P) were measured by an automatic weather station. During field sampling 243 at each site, we measured the hydrographical properties at 20 cm depth, including 244 water temperature (T_W) , pH, DO, and salinity. The detection limit and relative 245 standard deviations were ± 0.2 °C and ≤ 1.0 % for *Tw*, 0.01 and ≤ 1.0 % for pH, 0.1 mg 246 L⁻¹ and ≤2.0% for DO, and 0.1 ppt and ≤1.0% for salinity, respectively. Chlorophyll *a* 247 (Chl-*a*) was measured using a spectrophotometer (Shimadzu UV-2450, Japan) 248 following the method of Yang et al. $(2017b)$. The nitrite-nitrogen $(NO₃-N)$ and 249 ammonium-nitrogen (NH_4^+N) concentrations were determined using a continuous 250 flow injection analyzer (Yang et al., $2021a$). The detection limits for NO₃-N and 251 NH₄⁺-N were 0.6 μ g L⁻¹ and 0.6 μ g L⁻¹, respectively, and the relative standard 252 deviations were $\leq 2.0\%$ and $\leq 3.0\%$, respectively.

253 *2.6. Calculation of the carbon budget*

254 The carbon budgets of the coastal earthen aquaculture ponds were calculated 255 based on the mass balance (Flickinger et al., 2020; Zhang et al., 2020b) as follows:

$$
1W_{in} + RW_{in} + CA_{in} + PP_{in} + FA_{in} = RPS_{out} + HA_{out} + GCE_{out} + OW_{out} + SA_{out} + UC_{out} \quad (Eq.5)
$$

257 Among the input terms, IW_{in} is carbon input from the inflow water, RW_{in} is carbon 258 input through rainwater, CA_{in} is the amount of carbon in stocked shrimps, PP_{in} is 259 carbon input through phytoplankton primary production, FA_{in} is the amount of carbon 260 in the feed. Among the output terms, RPS_{out} is carbon loss via water column 261 respiration and sediment respiration, HA_{out} is the amount of carbon in harvested 262 shrimps, GCE_{out} is carbon loss through carbon greenhouse gas emissions $(CO₂$ and 263 CH₄), OW_{out} is carbon output from the ponds via outflow water, SA_{out} is sediment 264 carbon accumulation, and UC_{out} is the unaccounted portion (Flickinger et al., 2020).

265 Carbon input from each component $(IW_{in}$, RW_{in} , CA_{in} , PP_{in} and FA_{in}) was estimated as the product of the carbon concentrations and the total amount of each component. Carbon output through plankton respiration in the water column were 268 estimated as the product of the mean water depth, the *R*_P (Eq. 2), and the farming period (188 days). Carbon output via sediment respiration was determined as the product of the SOD (Eq. 3) and the farming period (188 days). Carbon output via HAout were estimated as the product of the total harvested shrimp biomass and the 272 carbon content of the shrimp. Carbon output via GCE_{out} across the WAI was estimated 273 as the product of the mean carbon greenhouse gas $(CO₂$ and $CH₄)$ fluxes, the pond 274 surface area (ha), and the aquaculture period (188 days). Carbon output via $\rm{OW_{out}}$ were estimated as the product of the total amount of water discharged and the carbon concentration in the discharged water. Carbon output via SAout was determined as the product of the total amount of sediment and the change in sediment carbon contents.

The environmental loading of carbon $(C_{EL}, \text{ kg } C t^{-1})$ of the cultured shrimp was 279 estimated as follows:

$$
C_{\rm EL} = \frac{C_{\rm E} - C_{\rm I}}{W_{\rm HA}}\tag{Eq.6}
$$

281 Where C_E is the total amount of carbon in the end of farming (kg), C_I is the total 282 amount of carbon at the initial stage of farming (kg) , and W_{HA} is the total weight of 283 harvested shrimps (t).

284 *2.7. Statistical analysis*

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Two–way analysis of variance (ANOVA) was performed to analyse the impacts of sampling ponds (Ponds I, II, and III), and time on carbon gas fluxes and total CO2-eq emissions, with sampling sites within ponds specified as a random variable. ANOVA was also used to test for the significant differences in hydrographical properties between ponds. Pearson correlation analysis (PCA) was performed to 290 analyse the correlations between carbon greenhouse gas emissions $(CO₂$ and $CH₄)$ and environmental parameters. Redundancy analysis (RDA) was performed to evaluate which environmental parameters would best explain the variability in carbon 293 greenhouse gas fluxes, with T_A , W_S , A_P , T_W , DO, pH, salinity, TOC, NH₄⁺-N, NO₃⁻-N, and Chl-*a* being included in the analysis. ANOVA and RDA were performed using SPSS 17.0 (SPSS Inc., USA) and the CANOCO 5.0 (Microcomputer Power, Ithaca, 296 USA), respectively. Results were summarized as "mean \pm 1 standard error (SE)" and 297 the significant level was set at $p = 0.05$. Sampling site map, conceptual diagrams and statistical plots were created using ArcGIS 10.2 (ESRI Inc., Redlands, CA, USA), EDraw Max 7.3 (EdrawSoft, Hong Kong, China), and OriginPro 9.0 (OriginLab Corp.

300 USA), respectively.

301

302 **3. Results**

303 *3.1. Water and sediment properties*

304 Hydrographical properties of the three ponds during the farming period are 305 shown in Figure 2. There were no significant differences in average T_w , DO, pH, 306 salinity and sediment TC content were observed among the shrimp ponds (ANOVA, 307 *p*>0.05; Figure 2a-d). However, significant differences were found in average total 308 dissolved organic carbon (TOC; Figure 2e) and chlorophyll *a* (Chl-*a*; Figure 2f) 309 among the ponds. TOC concentration in Pond II (30.9 \pm 3.1 mg L⁻¹) was significantly 310 higher than that in Pond I (20.9 \pm 1.4 mg L⁻¹) and Pond III (24.3 \pm 2.3 mg L⁻¹) 311 (*p*>0.05; Figure 2e). The mean Chl-*a* concentration was also significantly higher in 312 Pond II (146.9 \pm 15.1 μg L⁻¹), followed by Pond I (125.4 \pm 12.8 μg L⁻¹) and Pond III (116.1 \pm 10.2 µg L⁻¹) ($p < 0.05$) (Figure 2f). The sediment accumulation rate across three ponds over the farming period ranged from 0.83 to 0.84 cm month⁻¹ (average 0.83 ± 0.01 cm month⁻¹). The sediment TC content across three ponds increased from 316 an average of 16.2 ± 0.2 g kg⁻¹ at the beginning of the farming period to 18.6 ± 0.1 g 317 kg⁻¹ at the end of the farming period.

318 *3.2. Carbon greenhouse gas fluxes across the WAI*

Across all sampling dates and sites, net CO_2 flux ranged from -6.8–5.3 mg C m⁻² 320 h^{-1} in Pond I, -8.0–5.3 mg C m⁻² h⁻¹ in Pond II and -5.8–5.8 mg C m⁻² h⁻¹ in Pond III 321 (Figure 3a), with negative values indicating $CO₂$ uptake. On average, the net $CO₂$ flux

3.3. Carbon budget of the shrimp ponds

Table 1 shows the carbon inputs into the ponds. Primary production by 332 phytoplankton (269.1–327.6 g C m⁻²) was the largest component, accounting for 58.5–61.8% of the total input (Figure 5). Feed was the second largest component (144.9–187.3 g C m⁻²) that accounted for 31.9–35.3% of the total input (Figure 5). Stocked shrimps, rainwater and inflow water were only minor components of the carbon budget, representing on average 0.004%, 0.4–0.5%, and 4.9–5.8% of the total input, respectively (Figure 5).

The carbon outputs of the shrimp ponds are listed in Table 2, with their relative percentages of the total output shown in Figure 5. During the farming period, the main 340 output component was plankton respiration $(231.7–243.4 \text{ g C m}^{-2})$, which accounted for 44.0–53.6 % of the total output. Sediment accumulation $(82.0-117.6 \text{ g C m}^2)$ as the second largest component represented 18.0–21.7 % of the total output. Outflow water and biomass harvesting accounted for respectively 9.2–11.7 % and 9.0–11.4 % of the total output. Net carbon greenhouse gas emissions across the water-air interface and sediment respiration were only minor components, equivalent to 0.8–1.6 % and 0.01–0.03 % of the total output, respectively.

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- *3.4. Total CO2-equivalent emission from the shrimp ponds*

The combined carbon emission ($CO₂ + CH₄$), expressed in mg CO₂-eq m⁻² h⁻¹ based on GWP20, was 221.6±61.3 in Pond I, 885.7±221.4 in Pond II, and 478.9±229.7 in Pond III (Figure 4c). Combining data from the three ponds, the mean total CO₂-equivalent emission was 528.4±193.3 mg CO₂-eq m⁻² h⁻¹. Water-to-air CH₄ emission was the principal contributor of the total $CO₂$ -equivalent emission, with the largest emissions observed in August–September (Figures 3c).

3.5. Effects of environmental variables on carbon greenhouse gas fluxes

Based on Pearson correlation analysis, the CO2 fluxes were positively correlated 356 with T_w , pH, TOC and NH₄⁺-N, and negatively correlated with DO and Chl- a (p <0.01 357 or ≤ 0.05 ; Table 3). CH₄ fluxes were positively correlated with T_W and TOC, but 358 negatively correlated with DO $(p<0.01$ or <0.05 ; Table 3). According to the RDA results (Figure 6), Chl-*a*, TOC, and pH were the

- 360 significant factors driving the variations in $CO₂$ and $CH₄$ fluxes ($p<0.05$). Among them, Chl-*a* had the highest explanatory power (51.4%), followed by TOC (30.4%) 362 and pH (6.2%) .
- **4. Discussion**

4.1. Carbon budget of the aquaculture ponds

Primary production by phytoplankton was a main pathway for $CO₂$ uptake and

comprised 60% of the total carbon input, which was comparable to that observed in fish aquaculture ponds (46–78 %; Zhang et al., 2016, 2020c). The estimated mean 368 gross primary production (293 g C m⁻²) was higher than water-column respiration (238 g C m⁻²), causing a net autotrophic carbon fixation of ca. 55 g C m⁻² via photosynthesis. The other main carbon input came in the form of feeds (34%; 165 g C m^{-2}). In contrast to other studies (Flickinger et al., 2020; Guo et al., 2017; Zhang et al., 2020b), inflow water accounted for only a small percentage of the carbon input in the 373 present study (5%; 26 g C m⁻²), largely because of the low carbon concentration in the source water. Carbon input from rainwater was negligible.

Of the total excess carbon (i.e., net autotrophic carbon fixation + feed + 376 inflow/rain water; ca. 248 g C m⁻²), only ca. 21% was converted to shrimp biomass (51 g C m^2) , showing the low efficiency of *L. vannamei* in assimilating the feeds and utilizing the carbon input for growth (cf. Flickinger et al., 2020; Zhang et al., 2016, 379 2020b). Sedimentation and outflow together (151 g C m⁻²) accounted for another 61% of the total carbon output, in line with the range reported previously (Alongi et al., 2000, 2009; Sahu et al., 2013a, 2013b). Yet, net CO_2 and CH₄ emissions (13 g C m⁻²) removed only 5% of the excess carbon, similar to that observed in other semi-intensive and intensive aquaculture systems (Flickinger et al., 2020). The higher CO2 and CH4 emissions observed in August-September were likely due to the higher water temperature that not only increased respiration and methanogenesis but also decreased gas solubility in the water. On average, 8.0% (range 4.2–12.6%) of the excess carbon was unaccounted for. In addition to uncertainty associated with each of the measured input and output terms, some of the missing carbon was likely associated with respiratory activities by other heterotrophs and the loss of volatile organic carbon that was not measured in this study. Furthermore, carbon loss via denitrification (Hargreaves 1998), especially in nitrate-rich system, would not be 392 captured by our O_2 -based respiration measurements.

4.2. Spatio-temporal variations in carbon greenhouse gas emissions

 Large temporal variations in $CO₂$ and CH₄ fluxes have been found in various aquatic ecosystems, such as reservoirs (Gerardo-Nieto et al., 2017; Musenze et al., 2014), lakes (Natchimuthu et al., 2016; Xiao et al., 2021) and rivers (Luo et al., 2019; Zhao et al., 2013). However, comparable information is rare for aquaculture systems, particularly coastal earthen ponds (Chen et al., 2016; Zhang et al., 2022). Our results showed considerable temporal variations in the carbon greenhouse gas emissions from 400 three coastal earthen shrimp ponds (Figure 3a and $3b$). $CO₂$ and $CH₄$ emissions were higher in middle of the farming period (July–September) when water temperature tended to be higher than in the initial period (May–June) (Yang et al., 2021). Temperature can affect many abiotic and biotic parameters (e.g., plankton primary production, respiration, microbial activity, and nutrient availability, etc.) (Xiao et al., 2021) that in turn govern greenhouse gas production and consumption (Davidson et al., 2018; Kosten et al. 2012; Marotta et al., 2014; Rosentreter et al., 2017). Strong 407 correlations between $CO₂$ (or CH₄) emissions and water temperature were observed in this study and other studies (Shaher et al., 2020; Wu et al., 2018; Zhang et al., 2022), indicating that temperature plays an important role in driving the temporal change in carbon greenhouse gas emissions from the coastal earthen aquaculture ponds.

411 Our results also showed substantial between-pond differences in $CO₂$ and $CH₄$ 412 emissions (Figure 3a and 3b), with the lowest $CO₂$ and highest CH₄ emissions from Pond II. These variations are likely related to the differences in the physico-chemical properties of the sediment and overlying water in the ponds that influence greenhouse gas production and consumption. Among the hydrographical properties examined, only water TOC (Figure 2e), Chl-*a* (Figure 2f) and DIN (Yang et al., 2021a) differed 417 significantly among the ponds $(p<0.05$ or $\leq 0.01)$, with the highest values observed in Pond II. An earlier study at the same site reported significantly lower shrimp survival rate (55%) and higher feed conversion rate (2.6) in Pond II than in Ponds I (65% and 1.4) and III (67% and 1.6) (Yang et al., 2021b), which might have led to the accumulation of organic matter and phytoplankton in Pond II. The high abundance of phytoplankton in Pond II, as indicated by its higher Chl-*a*, would have allowed a stronger CO2 drawdown via photosynthesis (Davies et al., 2003; Xiao et al., 2021), 424 and subsequently a lower net $CO₂$ emission from this pond. Meanwhile, the higher organic matter accumulation at Pond II could have contributed to a higher CH⁴ production and emission (Davidson et al., 2018; Yang et al., 2020; Zhu et al., 2016). Despite the lack of data on the rates of microbial greenhouse gas production and consumption, the significant correlation between CO2 emission and Chl-*a*, and between CH4 emission and TOC (*p*<0.01; Table 3), implied that between-pond changes of CO2 and CH4 emissions were primarily driven by the availability of phytoplankton and carbon substrate (Figure 6).

433 The average water-to-air emissions of $CO₂$ and $CH₄$ during the farming period from our ponds were within the ranges observed elsewhere (Table 4; Zhu et al., 2016; Soares and Henry-Silva, 2019; Yang et al., 2018). Although CO2 and CH4 emissions comprised only a small proportion of the carbon budget, the strong global warming potential of these two gases especially that of CH4 implied that shrimp ponds could 438 still exert a considerable impact on the climate. The estimated total $CO₂$ -equivalent emission from the shrimp ponds averaged 528.4 ± 193.3 mg CO₂-eq m⁻² h⁻¹, which was much higher than that reported for lakes and reservoirs in China (104.0 and 61.1 $mg CO₂$ -eq m⁻² h⁻¹, respectively; Li et al., 2018) and around the world (Bastviken et al., 2011; Deemer et al., 2016), but comparable to some eutrophic lakes (Sun et al., 2021; Xing et al., 2005). Assuming that our data together with the literature data 444 (Table 4) were representative of global aquaculture ponds $(110,832 \text{ km}^2; \text{Verdegem})$ and Bosma, 2009), we estimated that aquaculture ponds would emit approximately 446 3.4×10⁵ Gg CO₂ y⁻¹ and 4.0×10⁴ Gg CH₄ y⁻¹ into the atmosphere. The corresponding 447 total CO₂-equivalent emission would be 3.7×10^6 Gg CO₂-eq y⁻¹, with CH₄ as the main contributor (91%).

The growing demand for animal proteins has prompted the intensification of livestock production and aquaculture, which has raised huge concerns over their environmental impacts including greenhouse gas emissions (Godfray and Garnett, 2014). Based on our estimation, aquaculture ponds contributed only ca. 1% of the global anthropogenic CH4 emission (Yuan et al., 2019). This was consistent with the

findings of a recent meta-analysis that aquaculture has a lower greenhouse gas emission than livestock production because of the absence of enteric fermentation (a major CH4 source in livestock) and a lower feed conversion ratio in the former (MacLeod et al., 2020).

Agriculture and livestock production are well-known sources of CH4, 459 contributing to about 40% of the anthropogenic CH₄ emission (Smith et al., 2008). Nevertheless, the nature and magnitude of CH4 emission from food production systems may change as the aquaculture sector continues to expand. Based on our data, we found that the magnitude of CH4 emission per unit area was substantially higher in aquaculture ponds (Yang et al., 2018; Yuan et al., 2021; Zhao et al., 2021) than in some agro-ecosystems such as paddy fields (e.g., Hao et al., 2016; Hou et al., 2010; Wu et al., 2018) and rice–wheat cropping systems (Guo et al., 2021; Wu et al., 2019; Yao et al., 2013), but comparable to rice-fish farming systems (e.g, Frei and Becker, 2005, Wang et al., 2019) except in India (e.g., Bhattacharyya et al., 2013; Datta et al., 2009) (Table 4). Compared to other agro-ecosystems, the large CH4 flux observed in aquaculture ponds may be the result of high sediments organic matter and the continuously flooded environment that would favor CH4 production and ebullition (Davidson et al., 2018; Yang et al., 2020).

Based on the results of the carbon budget, we could identify possible ways to reduce the climate footprint of aquaculture. Given that only 21% of the excess carbon was converted to shrimp biomass, the majority of the excess carbon would end up in various parts of the ponds (e.g. surface water) and adjacent ecosystems. By improving feed formulation and feed management, shrimp farmers could decrease the feed conversion ratio, increase the production efficiency and minimize waste generation (White, 2013). We also found that a large proportion (20%) of the excess carbon eventually accumulated in the sediment, which could promote anoxia and methanogenesis if left untreated. Sediment removal between farming seasons by dredging is not a common practice among the local shrimp farmers, but it could be a simple and effective strategy to mitigate greenhouse gas emissions, with the added 483 benefits of utilizing the organic-rich sediment as fertilizers (Pouil et al., 2019).

4.4. Limitation and future outlook

There were some limitations in our study. Firstly, we examined the carbon budget of shrimp ponds located in one estuary only during the farming period. Scaling up our data from the local to the regional scale may increase the uncertainty of budget calculation. To further improve the carbon budget accuracy at the regional and global scales, more studies on other variables such as aquaculture operation types, farmed species and management practice, are required. Secondly, this study only considered 491 the major gains and losses of carbon. However, \sim 4.2–12.6% of the carbon output was missing from the budget (Figure 5), which can be attributed to a combination of uncertainty associated with the measurements and carbon loss processes that were not captured by our methods. Some studies have shown that farmed animals' respiration could account for approximately 1.3–3.6% of the carbon loss (Xia et al., 2013; Zhang ; periphyton respiration is another potential contributor of carbon output (Zhang 497 et al., 2016). However, these processes can be patchy and may not be properly

captured by floating chamber measurements. Future studies should consider quantifying the respiration by the farmed animals and periphyton. Anaerobic respiration, which can be important in nitrate-rich aquaculture ponds (Hargreaves), can be better quantified by direct $CO₂$ measurements. Lastly, we measured carbon greenhouse gas emissions only during the daytime, whereas the diel variations of gaseous carbon fluxes might introduce some uncertainties to our carbon budget. Meanwhile, some pond management practices such as aeration and drainage activities could affect carbon emissions from aquaculture ponds (Datta et al., 2009; Kosten et al., 2020). Our present study was limited to the farming period when aeration was routinely applied to the ponds; therefore, the carbon emission measurements may not be representative of the situation where aeration is not used. To obtain accurate estimates of annual emissions, more detailed investigation of carbon emission during the dry-period (or non-farming period) and in non-aerated system/ period is needed (Kosten et al., 2020).

5. Conclusion

The present study adopted a carbon budget approach to investigate the major inputs and outputs of carbon in three coastal aquaculture ponds with *L. vannamei* in the MRE in southeastern China. In situ plankton production and respiration were the 517 main components of the carbon flows. Overall, water-to-air $CO₂$ and CH₄ emissions were relatively small contributions to the carbon budget, but the CH4 emission was still higher than that in other agro-ecosystems. We showed that the use of a mass

520 balance approach can provide useful insights into the carbon budget and dynamics 521 within the aquaculture ponds and help identify ways to improve production efficiency 522 and reduce the climate footprint of aquaculture production.

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Table 1
Carbon inputs (g C m⁻²) into the three aquaculture ponds with *Litopenaeus vannamei* during the farming period. Carbon inputs $(g \text{ C m}^{-2})$ into the three aquaculture ponds with *Litopenaeus vannamei* during the farming period.

 $\overline{1}$ \overline{c}

Carbon outputs ($g \text{ C m}^{-2}$) from the three aquaculture ponds with *Litopenaeus vannamei* during the farming period. Carbon outputs ($g \text{ C m}^{-2}$) from the three aquaculture ponds with *Litopenaeus vannamei* during the farming period. $\overline{4}$

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Table 2

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Correlation coefficient matrix of carbon greenhouse gas fluxes (CO₂ and CH₄) and different environmental variables for the coastal

Correlation coefficient matrix of carbon greenhouse gas fluxes (CO₂ and CH₄) and different environmental variables for the coastal

Table 4 \equiv

Comparison of CO2, CH4 and CO2-equivalent (based on GWP20) emission fluxes in different agro-ecosystems (e.g., aquaculture ponds, rice-fish farming Comparison of CO2, CH4 and CO2-equivalent (based on GWP20) emission fluxes in different agro-ecosystems (e.g., aquaculture ponds, rice-fish farming 12

Figure 1. Location of the research areas (a, b) and sampling sites (c) within Shanyutan

Wetland of the Min River Estuary (MRE) in Fujian, Southeast China.

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lowercase letters above the bars show significant differences between sampling ponds ($n = 75$; $p < 0.05$). lowercase letters above the bars show significant differences between sampling ponds (*n* = 75; *p*<0.05).

9 **Figure 3.** Temporal data of (a) CO₂ flux, (b) CH₄ flux, and (c) Total CO₂-eq flux (*T*_{Fluxes}) 10 based on GWP20, in the three shrimp aquaculture ponds (*Litopenaeus vannamei*) during 11 the farming period (May to October). Error bars represent standard error $(n = 5$ sites).

- **Figure 4.** Variations in the (a) CO₂ flux, (b) CH₄ flux, and (c) Total CO₂-eq flux (*T*Fluxes) based on GWP₂₀, in the three shrimp aquaculture ponds during the farming period (May to October). Different lowercase letters above the bars show significant differences between ponds during the farming period (May to October). Different lowercase letters above the bars show significant differences between 13 14
	- sampling ponds ($n = 75$; $p<0.05$). sampling ponds $(n = 75; p<0.05)$. 15

Figure 5. Percentages of carbon input and output components in the three shrimp aquaculture ponds (*Litopenaeus vannamei*) in the Min River Figure 5. Percentages of carbon input and output components in the three shrimp aquaculture ponds (Litopenaeus vannamei) in the Min River 17

Estuary during the farming period (May to October). Estuary during the farming period (May to October). 18

20 **Figure 6.** Redundancy analysis (RDA) biplots of the relationships between $CO₂$ and CH4 fluxes and environmental variables (meteorological and hydrographical). The loadings of environmental factors (arrows) and the scores of observations in all sampling campaign are presented. *A*P, *T*A, *W*S, *T*W, TOC, DO, and Chl-*a* represent atmospheric pressure, air temperature, wind speed, water temperature, total organic carbon, dissolved oxygen, and chlorophyll *a*, respectively. The pie chart shows the explanatory power of the different environmental factors.