1	Insights into the farming-season carbon budget of coastal earthen
2	aquaculture ponds in southeastern China
3	Ping Yang ^{a,b,c,*} , Kam W. Tang ^d , Hong Yang ^e , Chuan Tong ^{a,b,c,*} , Nan Yang ^a ,
4	Derrick Y. F. Lai ^f , Yan Hong ^a , Manjing Ruan ^a , Yingying Tan ^a , Guanghui Zhao ^a ,
5	Ling Li ^a , Chen Tang ^a
6	^a School of Geographical Sciences, Fujian Normal University, Fuzhou 350007, P.R. China,
7	^b Key Laboratory of Humid Subtropical Eco-geographical Process of Ministry of Education,
8	Fujian Normal University, Fuzhou 350007, P.R. China,
9	Research Centre of Wetlands in Subtropical Region, Fujian Normal University, Fuzhou 350007,
10	P.R. China,
11	^d Department of Biosciences, Swansea University, Swansea SA2 8PP, U. K.
12	^e Department of Geography and Environmental Science, University of Reading, Reading, RG6 6AB,
13	UK
14	^f Department of Geography and Resource Management, The Chinese University of Hong Kong,
15	Hong Kong, China
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20	*Correspondence to:
21	Ping Yang (yangping528@sina.cn), Chuan Tong (tongch@fjnu.edu.cn)
22	Telephone: 086-0591-87445659 Fax: 086-0591-83465397

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23 **ABSTRACT**

Small-hold aquaculture ponds are widespread in China, but their carbon greenhouse 24 25 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 26 China with the whiteleg shrimp (Litopenaeus vannamei) during the farming period. 27 28 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 29 outputs occurred through planktonic respiration (44.0-53.6%) and sedimentation 30 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 32 CO₂-equivalent emission of 528.4 \pm 193.3 mg CO₂-eq m⁻² h⁻¹ based on GWP₂₀. We 33 34 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 35 substrate supply. Nevertheless, the magnitude of CH₄ emission from these ponds was 36 still higher than some other agro-ecosystems. Moreover, we found that only 21% of 37 the excess organic carbon was converted to shrimp biomass, while another 20% ended 38 up in the sediment. Our findings suggested that lowering the feed conversion ratio and 39 removing the bottom sediments regularly could help improve production efficiency, 40 reduce the excessive accumulation of carbon-rich detritus and minimize the climatic 41 warming impacts of aquaculture production. 42

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

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List of abbreviations:

46	A _P : Atmospheric Pressure	CH4: Methane
47	Chl-a: Chlorophyll a	CO2: Carbon dioxide
48	DO: Dissolved Oxygen	DOC: Dissolved Organic Carbon
49	GCE: Gaseous carbon (CO ₂ and CH ₄) emis	ssion
50	GWP ₂₀ : Global Warming Potential (20-yea	r time horizon)
51	HA: Harvested Animals	IW: Inflow Water
52	OW: Outflow Water	PP: Primary Production
53	PR: Water-column respiration	POC: Particulate Organic Carbon
54	SA: Sediment Accumulation	SR: Sediment Respiration
55	SOD: Sediment Oxygen Demand	<i>T</i> _A : Air Temperature
56	TC: Total Carbon	<i>T</i> w: Water Temperature

*W*s: Wind Speed

58 **1. Introduction**

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Since the Industrial Revolution, the atmospheric concentrations of the two main 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 67 world-wide, especially small-hold aquaculture ponds (FAO, 2018; Ren et al., 2019), 68 69 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 70 aquaculture ponds can have high water-to-air CO₂ and CH₄ emissions, owing to their 71 high productivities and shallow water depths (MacLeod et al., 2020; Yuan et al., 72 2019). Although there have been some efforts to characterize CO₂ and CH₄ fluxes 73 74 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), 75 relevant data are still scarce for China, where small-hold aquaculture ponds are 76 77 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 78 small-hold aquaculture ponds, may divert the carbon downstream. On the other hand, 79

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).

85 In the current study, we determined the different carbon input and output components of three earthen aquaculture ponds with Litopenaeus vannamei within a 86 subtropical estuary in southeastern China. The research main objectives to: (1) assess 87 88 the carbon budgets of the earthen shrimp ponds, (2) quantify the contribution of CO_2 and CH₄ emissions to the total carbon output, and (3) evaluate the role of aquaculture 89 ponds in driving global warming. We hypothesized that CO₂ and CH₄ emissions 90 91 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 92 recommendations to improve the production efficiency and minimize the climate 93 footprint of shrimp aquaculture. 94

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- 96 2. Materials and methods
- 97 *2.1. Study area*

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 annual average precipitation of 1,350 mm and air temperature of 19.6 °C (Tong et al., 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.

107 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 the farming period (Yang et al., 2021a). The farming period is usually between 5th 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 115 MRE. The seawater was first passed through a 2 mm mesh bag to exclude predators 116 and competitors. After seven days, trichloroisocyanuric acid (~25 kg pond⁻¹) was 117 added to disinfect the pond water, followed by the addition of calcium oxide lime (0.5 118 t ha⁻¹) and calcium superphosphate fertilizer (1.5–2.0 kg per 1000 m³). Before shrimp 119 stocking, probiotics (200 mL ha⁻¹) were added, and water conditions (e.g., alkalinity 120 or acidity, salinity, etc.) were checked to make sure they were in the correct range. 121 Each earthen pond was stocked with L. vannamei at a density of 215 post larvae m^{-2} . 122 Commercial feed pellets were added daily during the farming period. Aeration was 123

124 provided by aerators in the ponds. After harvesting, the pond water was discharged.

125 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.

133 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 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 deviations were 4 μ g L⁻¹ and \leq 1.0% for TC, respectively.

140 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 ; mg O₂ L⁻¹) were calculated from the changes in DO concentrations as follows (Zhang et al., 2016):

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

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

where DO_L (mg L⁻¹) and DO_D (mg L⁻¹) are the final DO concentrations in the light and dark bottles, respectively; DO_I (mg L⁻¹) is the initial dissolved oxygen. Measurements were converted to carbon using the conversion of 1 mg O₂ to 0.375 mg C (Guo et al., 2017; Winberg, 1980).

157 *2.3.3. Input: Inflow water and rainwater*

Samples of inflow water were collected from two inlets using an organic glass 158 hydrophore at each pond, while rainwater was sampled by a rain gauge 5 times during 159 160 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 161 of the water sample was filtered through a pre-combusted 0.45 µm glass fibre filter to 162 separate the particulate organic carbon (POC) and dissolved organic carbon (DOC) 163 fractions. Both fractions were subsequently analyzed using a total organic carbon 164 (TOC) analyzer (TOC-V_{CPH/CPN}, Shimadzu, Japan). In addition, rainfall data from a 165 local weather station were used to estimate the total precipitation entering each pond 166 during the farming period. 167

Respiration in the water column by both autotrophic and heterotrophic plankton 169 170 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 171 172 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 173 (diameter 6 cm). The sediment samples were sealed in vacuum inside the incubation 174 chambers. Both sediment and pond water samples were transported to the laboratory 175 176 within 4 hr, and then were allowed to equilibrate to the lab condition for 2 h (Zhang et al., 2016). The incubation chamber was filled with pond water up to 15 cm above the 177 surface of sediment, and then sealed with a Teflon stopper. The incubation was done 178 179 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 180 chamber to determine DO by Winkler titration. Incubation chambers filled with pond 181 182 water only (no sediment) was used as the control. Sediment oxygen demand (SOD, mg $O_2 m^{-2} d^{-1}$) was determined from the change in DO in the overlying water: 183

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$$SOD = \frac{(DO_{I} - DO_{F}) \times V_{OW}}{A_{SC} \times T_{IE}}$$
(Eq.3)

where DO_I and DO_F (mg O₂ L⁻¹) are the initial and final DO concentrations, respectively; V_{OW} (L) is the volume of overlying water in the incubation chambers, A_{SC} (m²) is the cross-sectional area of the sediment core, T_{IE} (h) is the duration of the incubation experiment. SOD (mg O₂ m⁻² h⁻¹) was converted to carbon demand (mg C m⁻² d⁻¹) (1 mg O₂ = 0.375 mg C) according to Winberg (1980). 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 196 sedimentation was derived from sediment height accumulation over time and 197 sediment carbon content. Briefly, five 0.5 m² ceramic tiles were placed inside each of 198 the ponds at the start of the farming period, and the height of the accumulated 199 sediment (cm) on the tiles at the end of the farming period was measured by vernier 200 201 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 202 laboratory, the sediment TC were determined using an Elementar combustion analyzer 203 (Elementar Vario MAX CN, Germany). The accumulated sediment height and 204 sediment TC content were then used to estimate the total amount of carbon 205 sedimentation throughout the farming period (Flickinger et al., 2020). 206

207 2.3.6. Output: Carbon greenhouse gas emissions

The fluxes of CO_2 and CH_4 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 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 212 collected from the floating chamber air headspace at 0, 15, 30, and 45 min, and were 213 214 then transferred into sample bags. Sampling was done between 9:00 and 11:00 local time outside of the time when the aerators were running. CO₂ and CH₄ concentrations 215 in the gas sampled were analysed within 24 h by a gas chromatograph equipped with 216 a flame ionization detector (GC-2010, Shimadzu, Kyoto, Japan). CH₄ fluxes 217 determined by the floating chamber method represented the sum of ebullitive CH₄ 218 fluxes and diffusive CH₄ fluxes (Wu et al., 2019; Zhu et al., 2016). Gas fluxes (CO₂ or 219 CH₄; mg C m⁻² h⁻¹) across the WAI were estimated as the rate of change in the mass of 220 CO₂ (or CH₄) per unit surface area per unit time (Yuan et al., 2021; Zhu et al., 2016) 221 as follows: 222

$$F = \frac{\mathrm{d}c}{\mathrm{d}t} \bullet \frac{M_{\mathrm{M}}}{V_{\mathrm{M}}} \bullet \frac{P}{P_{0}} \bullet \frac{T}{T_{0} + T} \bullet H$$
(Eq. 4)

where *F* is the fluxes of CO₂ or CH₄ (mg C m⁻² h⁻¹); d*c*/d*t* is the slope of the CO₂ (or CH₄) concentration (*c*, mmol mol⁻¹) curve variation over time (*t*, hour); $M_{\rm M}$ is the molar mass of CO₂ or CH₄ (mg mol⁻¹); $V_{\rm M}$ is the gas molar volume (m³ mol⁻¹); P_0 and T_0 is the atmospheric pressure (kPa) and absolute temperature (K), respectively, under the standard condition; *P* and *T* is the air pressure (kPa) of the sampling pond and the air temperature (K) during the measurement, respectively; *H* is the floating chamber 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 effects are often expressed as CO_2 -equivalent on a chosen time horizon by applying the appropriate global warming potential values (Skytt et al., 2020). In the present study, we multiplied the mass of CH₄ by a global warming potential (GWP₂₀) value of 84 to calculate its CO₂ equivalent (CO₂-eq) on a 20-year time horizon (Fang et al., 2021; IPCC, 2014). This was then added to the mass of CO₂ emission to calculate the total CO₂-eq emission on a 20-year time horizon.

240 2.5. Measurements of ancillary environmental variables

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

253 2.6. Calculation of the carbon budget

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

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

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

Carbon input from each component (IWin, RWin, CAin, PPin and FAin) was 265 estimated as the product of the carbon concentrations and the total amount of each 266 267 component. Carbon output through plankton respiration in the water column were estimated as the product of the mean water depth, the R_P (Eq. 2), and the farming 268 period (188 days). Carbon output via sediment respiration was determined as the 269 270 product of the SOD (Eq. 3) and the farming period (188 days). Carbon output via 271 HAout were estimated as the product of the total harvested shrimp biomass and the 272 carbon content of the shrimp. Carbon output via GCEout across the WAI was estimated as the product of the mean carbon greenhouse gas (CO₂ and CH₄) fluxes, the pond 273 surface area (ha), and the aquaculture period (188 days). Carbon output via OW_{out} 274 were estimated as the product of the total amount of water discharged and the carbon 275 276 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. 277

278 The environmental loading of carbon (C_{EL} , kg C t⁻¹) of the cultured shrimp was 279 estimated as follows:

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

Where $C_{\rm E}$ is the total amount of carbon in the end of farming (kg), $C_{\rm I}$ is the total amount of carbon at the initial stage of farming (kg), and $W_{\rm HA}$ is the total weight of harvested shrimps (t).

284 2.7. Statistical analysis

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Two-way analysis of variance (ANOVA) was performed to analyse the impacts 285 of sampling ponds (Ponds I, II, and III), and time on carbon gas fluxes and total 286 CO₂-eq emissions, with sampling sites within ponds specified as a random variable. 287 ANOVA was also used to test for the significant differences in hydrographical 288 289 properties between ponds. Pearson correlation analysis (PCA) was performed to analyse the correlations between carbon greenhouse gas emissions (CO₂ and CH₄) and 290 environmental parameters. Redundancy analysis (RDA) was performed to evaluate 291 292 which environmental parameters would best explain the variability in carbon greenhouse gas fluxes, with T_A , W_S , A_P , T_W , DO, pH, salinity, TOC, NH₄⁺–N, NO₃⁻–N, 293 and Chl-a being included in the analysis. ANOVA and RDA were performed using 294 SPSS 17.0 (SPSS Inc., USA) and the CANOCO 5.0 (Microcomputer Power, Ithaca, 295 USA), respectively. Results were summarized as "mean ± 1 standard error (SE)" and 296 the significant level was set at p = 0.05. Sampling site map, conceptual diagrams and 297 298 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. 299

300 USA), respectively.

301

302 3. Results

303 *3.1. Water and sediment properties*

Hydrographical properties of the three ponds during the farming period are 304 shown in Figure 2. There were no significant differences in average T_w , DO, pH, 305 salinity and sediment TC content were observed among the shrimp ponds (ANOVA, 306 p>0.05; Figure 2a-d). However, significant differences were found in average total 307 dissolved organic carbon (TOC; Figure 2e) and chlorophyll a (Chl-a; Figure 2f) 308 among the ponds. TOC concentration in Pond II $(30.9 \pm 3.1 \text{ mg L}^{-1})$ was significantly 309 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) 310 (p>0.05; Figure 2e). The mean Chl-a concentration was also significantly higher in 311 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 312 $(116.1 \pm 10.2 \ \mu g \ L^{-1}) \ (p < 0.05)$ (Figure 2f). The sediment accumulation rate across 313 three ponds over the farming period ranged from 0.83 to 0.84 cm month⁻¹ (average 314 0.83 ± 0.01 cm month⁻¹). The sediment TC content across three ponds increased from 315 an average of 16.2 ± 0.2 g kg⁻¹ at the beginning of the farming period to 18.6 ± 0.1 g 316 kg⁻¹ at the end of the farming period. 317

318 3.2. Carbon greenhouse gas fluxes across the WAI

Across all sampling dates and sites, net CO₂ flux ranged from -6.8–5.3 mg C m⁻² 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 (Figure 3a), with negative values indicating CO₂ uptake. On average, the net CO₂ flux

322	was highest in Pond III ($1.7 \pm 0.9 \text{ mg C m}^{-2} \text{ h}^{-1}$), followed by Ponds I ($1.5 \pm 1.0 \text{ mg C}$
323	$m^{-2} h^{-1}$) and II (0.9 ± 1.1 mg C m ⁻² h ⁻¹) (Figure 4a). Net CH ₄ flux ranged 0.02–6.8,
324	0.2–28.1 and 0.2–39.8 mg C $m^{-2} h^{-1}$ in Ponds I, II and III (Figure 3b), respectively.
325	CH ₄ fluxes decreased significantly in the order of Pond II (7.9 \pm 1.9 mg C m ⁻² h ⁻¹) >
326	Pond III $(4.2 \pm 2.7 \text{ mg C m}^{-2} \text{ h}^{-1}) > \text{Pond I} (1.9 \pm 0.5 \text{ mg C m}^{-2} \text{ h}^{-1}) (p < 0.01; \text{Figure})$
327	4b). Carbon greenhouse gas emissions varied significantly with time ($p < 0.01$; Figure
328	3), with lower CO ₂ in May-June and lower CH ₄ emissions in May and October, where
329	higher emissions of both gases were observed in August and September.

330 *3.3. Carbon budget of the shrimp ponds*

Table 1 shows the carbon inputs into the ponds. Primary production by 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 output component was plankton respiration (231.7–243.4 g C m⁻²), which accounted for 44.0–53.6 % of the total output. Sediment accumulation (82.0–117.6 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.

347 *3.4. Total CO*₂*-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).

354 *3.5. Effects of environmental variables on carbon greenhouse gas fluxes*

Based on Pearson correlation analysis, the CO₂ fluxes were positively correlated with T_W , pH, TOC and NH₄⁺-N, and negatively correlated with DO and Chl-*a* (*p*<0.01 or <0.05; Table 3). CH₄ fluxes were positively correlated with T_W and TOC, but 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

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%) and pH (6.2%).

363 **4. Discussion**

364 *4.1. Carbon budget of the aquaculture ponds*

365 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 366 fish aquaculture ponds (46-78 %; Zhang et al., 2016, 2020c). The estimated mean 367 gross primary production (293 g C m⁻²) was higher than water-column respiration 368 (238 g C m⁻²), causing a net autotrophic carbon fixation of ca. 55 g C m⁻² via 369 photosynthesis. The other main carbon input came in the form of feeds (34%; 165 g C 370 371 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 372 present study (5%; 26 g C m⁻²), largely because of the low carbon concentration in the 373 source water. Carbon input from rainwater was negligible. 374

Of the total excess carbon (i.e., net autotrophic carbon fixation + feed + 375 inflow/rain water; ca. 248 g C m⁻²), only ca. 21% was converted to shrimp biomass 376 (51 g C m⁻²), showing the low efficiency of L. vannamei in assimilating the feeds and 377 utilizing the carbon input for growth (cf. Flickinger et al., 2020; Zhang et al., 2016, 378 2020b). Sedimentation and outflow together (151 g C m⁻²) accounted for another 61% 379 of the total carbon output, in line with the range reported previously (Alongi et al., 380 2000, 2009; Sahu et al., 2013a, 2013b). Yet, net CO₂ and CH₄ emissions (13 g C m⁻²) 381 removed only 5% of the excess carbon, similar to that observed in other 382 semi-intensive and intensive aquaculture systems (Flickinger et al., 2020). The higher 383 CO₂ and CH₄ emissions observed in August-September were likely due to the higher 384 water temperature that not only increased respiration and methanogenesis but also 385 decreased gas solubility in the water. On average, 8.0% (range 4.2-12.6%) of the 386 excess carbon was unaccounted for. In addition to uncertainty associated with each of 387

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 captured by our O₂-based respiration measurements.

393 4.2. Spatio-temporal variations in carbon greenhouse gas emissions

Large temporal variations in CO₂ and CH₄ fluxes have been found in various 394 aquatic ecosystems, such as reservoirs (Gerardo-Nieto et al., 2017; Musenze et al., 395 396 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, 397 398 particularly coastal earthen ponds (Chen et al., 2016; Zhang et al., 2022). Our results 399 showed considerable temporal variations in the carbon greenhouse gas emissions from three coastal earthen shrimp ponds (Figure 3a and 3b). CO₂ and CH₄ emissions were 400 higher in middle of the farming period (July-September) when water temperature 401 tended to be higher than in the initial period (May-June) (Yang et al., 2021). 402 Temperature can affect many abiotic and biotic parameters (e.g., plankton primary 403 404 production, respiration, microbial activity, and nutrient availability, etc.) (Xiao et al., 2021) that in turn govern greenhouse gas production and consumption (Davidson et 405 al., 2018; Kosten et al. 2012; Marotta et al., 2014; Rosentreter et al., 2017). Strong 406 correlations between CO₂ (or CH₄) emissions and water temperature were observed in 407 this study and other studies (Shaher et al., 2020; Wu et al., 2018; Zhang et al., 2022), 408 indicating that temperature plays an important role in driving the temporal change in 409

410 carbon greenhouse gas emissions from the coastal earthen aquaculture ponds.

Our results also showed substantial between-pond differences in CO₂ and CH₄ 411 412 emissions (Figure 3a and 3b), with the lowest CO₂ and highest CH₄ emissions from 413 Pond II. These variations are likely related to the differences in the physico-chemical 414 properties of the sediment and overlying water in the ponds that influence greenhouse 415 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 416 significantly among the ponds (p < 0.05 or < 0.01), with the highest values observed in 417 418 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 419 1.4) and III (67% and 1.6) (Yang et al., 2021b), which might have led to the 420 421 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 422 stronger CO₂ drawdown via photosynthesis (Davies et al., 2003; Xiao et al., 2021), 423 424 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 425 426 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 427 consumption, the significant correlation between CO2 emission and Chl-a, and 428 between CH_4 emission and TOC (p < 0.01; Table 3), implied that between-pond 429 changes of CO₂ and CH₄ emissions were primarily driven by the availability of 430 phytoplankton and carbon substrate (Figure 6). 431

The average water-to-air emissions of CO₂ and CH₄ during the farming period 433 434 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 435 436 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 437 still exert a considerable impact on the climate. The estimated total CO₂-equivalent 438 emission from the shrimp ponds averaged $528.4 \pm 193.3 \text{ mg CO}_2$ -eq m⁻² h⁻¹, which 439 was much higher than that reported for lakes and reservoirs in China (104.0 and 61.1 440 mg CO₂-eq m⁻² h⁻¹, respectively; Li et al., 2018) and around the world (Bastviken et 441 al., 2011; Deemer et al., 2016), but comparable to some eutrophic lakes (Sun et al., 442 443 2021; Xing et al., 2005). Assuming that our data together with the literature data (Table 4) were representative of global aquaculture ponds (110,832 km²; Verdegem 444 and Bosma, 2009), we estimated that aquaculture ponds would emit approximately 445 3.4×10^5 Gg CO₂ y⁻¹ and 4.0×10^4 Gg CH₄ y⁻¹ into the atmosphere. The corresponding 446 total CO₂-equivalent emission would be 3.7×10^6 Gg CO₂-eq y⁻¹, with CH₄ as the main 447 contributor (91%). 448

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 454 findings of a recent meta-analysis that aquaculture has a lower greenhouse gas 455 emission than livestock production because of the absence of enteric fermentation (a 456 major CH₄ source in livestock) and a lower feed conversion ratio in the former 457 (MacLeod et al., 2020).

Agriculture and livestock production are well-known sources of CH₄, 458 459 contributing to about 40% of the anthropogenic CH₄ emission (Smith et al., 2008). Nevertheless, the nature and magnitude of CH₄ emission from food production 460 systems may change as the aquaculture sector continues to expand. Based on our data, 461 462 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 463 some agro-ecosystems such as paddy fields (e.g., Hao et al., 2016; Hou et al., 2010; 464 465 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, 466 2005, Wang et al., 2019) except in India (e.g., Bhattacharyya et al., 2013; Datta et al., 467 468 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 469 continuously flooded environment that would favor CH₄ production and ebullition 470 471 (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 476 conversion ratio, increase the production efficiency and minimize waste generation 477 478 (White, 2013). We also found that a large proportion (20%) of the excess carbon eventually accumulated in the sediment, which could promote anoxia and 479 480 methanogenesis if left untreated. Sediment removal between farming seasons by 481 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 482 benefits of utilizing the organic-rich sediment as fertilizers (Pouil et al., 2019). 483

484 *4.4. Limitation and future outlook*

There were some limitations in our study. Firstly, we examined the carbon 485 budget of shrimp ponds located in one estuary only during the farming period. Scaling 486 487 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 488 scales, more studies on other variables such as aquaculture operation types, farmed 489 490 species and management practice, are required. Secondly, this study only considered the major gains and losses of carbon. However, $\sim 4.2-12.6\%$ of the carbon output was 491 492 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 493 captured by our methods. Some studies have shown that farmed animals' respiration 494 could account for approximately 1.3–3.6% of the carbon loss (Xia et al., 2013; Zhang 495 2019); periphyton respiration is another potential contributor of carbon output (Zhang 496 et al., 2016). However, these processes can be patchy and may not be properly 497

captured by floating chamber measurements. Future studies should consider 498 quantifying the respiration by the farmed animals and periphyton. Anaerobic 499 respiration, which can be important in nitrate-rich aquaculture ponds (Hargreaves 500 1998), can be better quantified by direct CO₂ measurements. Lastly, we measured 501 502 carbon greenhouse gas emissions only during the daytime, whereas the diel variations 503 of gaseous carbon fluxes might introduce some uncertainties to our carbon budget. Meanwhile, some pond management practices such as aeration and drainage activities 504 505 could affect carbon emissions from aquaculture ponds (Datta et al., 2009; Kosten et 506 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 507 be representative of the situation where aeration is not used. To obtain accurate 508 509 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 510 (Kosten et al., 2020). 511

512

513 **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 main components of the carbon flows. Overall, water-to-air CO_2 and CH_4 emissions were relatively small contributions to the carbon budget, but the CH_4 emission was still higher than that in other agro-ecosystems. We showed that the use of a mass balance approach can provide useful insights into the carbon budget and dynamics
within the aquaculture ponds and help identify ways to improve production efficiency
and reduce the climate footprint of aquaculture production.

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Pond	Inflow water	Rainwater	Stocked biomass	Primary production	Feed	Total
	26.40	2.27	0.02	280.80	144.90	454.39
Π	26.40	2.23	0.02	327.60	187.26	543.51
Π	26.40	2.23	0.02	269.10	162.29	460.04
Mean	26.40	2.24	0.02	292.50	164.82	485.98

Carbon inputs (g C m⁻²) into the three aguaculture bonds with *Litonengeus vannamei* during the farming period.

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Table 1

	aurpaus (5 0 m		and anominate bound				morred Summer		
P Q	Respiration		II	Water-to-8	air emission	91-0	C. J	Letel	
ronu	Water column	Sediment	- Harvesteu Diomass	CO_2	CH_4	- Outflow water	Seament accumulation	lotal	Ouners
Ι	243.36	0.03	40.89	1.5	1.9	53.26	81.98	422.92	31.47
Π	239.01	0.01	59.77	0.9	7.9	49.88	117.65	475.12	68.39
III	231.66	0.03	52.34	1.7	4.2	51.39	99.22	440.54	19.50
Mean	238.01	0.02	51.00	1.37	4.67	51.51	99.62	446.20	39.78

Carbon outputs (g C m⁻²) from the three aquaculture ponds with *Litopenaeus vannamei* during the farming period. 4

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Table 2

	CO ₂ flux CH ₄ flux	$T_{ m A}$	$A_{ m P}$	$W_{ m S}$	$T_{ m W}$	DO	рН	Salinity	TOC	NO3N	NH4 ⁺ -N	Chl-a
CO ₂ flux	1 0.273	060.0	0.015	-0.276	0.330*	-0.474**	0.406^{**}	-0.061	0.440 **	0.258	0.399**	-0.799**
CH4 flux	1	0.207	-0.127	-0.160	0.353*	-0.478**	0.078	-0.133	0.757**	0.134	-0.080	-0.036
$T_{ m A}$		1	-0.804**	0.181	0.894^{**}	-0.051	0.128	-0.184	0.104	-0.084	-0.532**	-0.034
$A_{ m P}$			1	-0.277	-0.781**	0.118	0.147	0.259	-0.094	0.257	0.619**	0.115
$W_{ m S}$				1	0.036	0.061	-0.109	0.018	-0.276	-0.203	-0.296*	0.056
$T_{ m W}$					1	-0.233	0.214	-0.308*	0.277	-0.037	-0.398**	-0.266
DO						1	-0.152	-0.032	-0.599**	-0.151	-0.069	0.457**
μd							1	0.045	0.129	0.326*	0.231	-0.129
Salinity								1	0.182	0.192	0.088	0.143
TOC									1	0.365*	0.037	-0.203
NO3NO										1	0.433**	-0.142
NH4 ⁺ -N											1	-0.259
Chl-a												1

and chlorophyll a, respectively.

Table 3

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Correlation coefficient matrix of carbon greenhouse gas fluxes (CO2 and CH4) and different environmental variables for the coastal 9

11 Table 4

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

systems, paddy fields and rice-wheat cropping systems). ND indicates no data. Numbers in parentheses are mean values. 13

-			CO ₂ flux	CH4 flux	CO2-eq flux	
Types	Location	Study period	(mg C m ⁻² h ⁻¹)	(mg m ⁻² h ⁻¹)	(mg CO2-eq m ⁻² h ⁻¹)	Keference
Aquaculture ponds	Min River Estuary, China	June–October 2015	$-8.0 - 5.8 \ (1.3)$	0.02 - 39.8 (4.7)	-22.3 - 4475.2 (528.4)	This study
	Jiulong River Estuary, China	June–October 2015	-2.7 –15.7 (4.2)	1.9 - 17.6 (7.2)	$203.7 - 2035.0 \ (825.2)$	Yang et al., 2018
	Zhangjiang River Estuary, China	January-December 2020	-84.2 -34.6 (-25.8)	$0.02 - 2.8 \ (0.2)$	— (-74.2)	Zhang et al., 2022
	Gaoqing, China	April-September 2013	$13.8 - 41.2 \ (26.6)$	ND	$50.6 - 150.9 \ (97.7)$	Chen et al., 2016
	Anhui Province, China	January-December 2016–2019	ND	$0.4-60.1\ (14.8)$	$33.6 - 5048.4 \ (1243.2)$	Zhao et al., 2021
	Shanghai, China	July–September 2013	$-9.6 - 100.4 \ (32.5)$	0.5 – 36.2 (7.0)	$32.4 - 3141.2 \ (620.5)$	Zhu et al., 2016
	Jiangsu province, China	August 2017–August 2019	ND	$0.8 - 1.0\ (0.9)$	$67.2 - 84.0 \ (75.6)$	Fang et al., 2021
	Northeastern, Brazil	ND	$-42.9 - 5.6 \ (18.6)$	-9.8 - 20.5 (5.3)	-1259.3 – 2309.7 (662.1)	Soares and Henry-Silva, 2019
	Suzhou, China	March 2013–March 2014	ND	$6.8 - 10.7 \ (8.2)$	757.7 – 1201.2 (923.2)	Yuan et al., 2021
	Ganyu County, China	ND	19.2 – 26.6 (5.5)	0.05 – 0.06 (ND)	75.5 - 103.5 (25.9)	Zhang et al., 2020
Rice-fish farming systems	Cuttack, India	July–December 2011	ND	0.7 - 10.6 (ND)	58.8 – 890.4 (ND)	Bhattacharyya et al., 2013
	ND	June to September 2003	ND	12.1 – 13.6 (12.9)	$1016.4 - 1142.4 \ (1083.6)$	Frei and Becker, 2005
	Cuttack, India	June–December 2005	ND	2.4 - 2.5 (2.5)	208.3 - 211.7 (210.0)	Datta et al., 2009
	Jiangjia village, northeast China	June–October 2013	ND	$0.3 - 23.7 \ (11.9)$	$25.2 - 1990.8 \ (999.6)$	Wang et al., 2019
Paddy fields	Xinghua, China	June 2014–June 2015	ND	$0.6 - 0.7 \ (0.66)$	50.4 - 58.8 (55.4)	Wu et al., 2018
	Yangtze River Delta, China	Rice seasons of 2005-2007	ND	-0.3 – 23.9 (ND)	-25.2 - 2007.6 (55.4)	Yao et al., 2012
	Taihu Lake region, China	June to October 2009 (2010)	ND	$0.6 - 3.0 \ (1.8)$	$50.4 - 251.1 \; (147.2)$	Hou et al., 2010
	Hunan province, China	Rice season in 2011 (2012)	ND	0.7 – 17.3 (ND)	58.8 – 1453.2 (ND)	Shen et al., 2014
	Chongqing, Southwest China	October 2009–October 2010	ND	$0.9 - 3.0 \ (1.7)$	75.6 – 252.0 (144.7)	Hao et al., 2016
Rice-wheat cropping systems	Taihu Lake District, China	January 2011 to November 2012	ND	-0.04 – 69.5 (ND)	3.4 - 5838.0 (ND)	Zhang et al., 2015
	Yangtze River Delta, China	June 2005 to June 2006	ND	-0.03-57.0 (2.5)	$2.5 - 4788.0 \ (211.6)$	Yao et al., 2013
	Hubei Province, China	June 2012 to June 2018	ND	-0.8-76.9 (4.1)	67.2 – 6459.6 (344.4)	Guo et al., 2021
	Jiangsu Province, China	June 2012 to May 2018	ND	$-0.4-1.0\ (0.7)$	33.6 - 84.0~(58.8)	Wu et al., 2019



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

3 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).



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Figure 3. Temporal data of (a) CO_2 flux, (b) CH_4 flux, and (c) Total CO_2 -eq flux (T_{Fluxes}) based on GWP₂₀, in the three shrimp aquaculture ponds (*Litopenaeus vannamei*) during 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 13 14
 - 15 sampling ponds (n = 75; p < 0.05).



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

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



Figure 6. Redundancy analysis (RDA) biplots of the relationships between CO_2 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.

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