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15 **Inland saline aquaculture increased carbon accumulation rate and stability in pond**  
16 **sediments under semi-arid climate**

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32

33 **Abstract**

34 **Purpose:** Similar to fresh- and brackish water aquaculture ponds, commercial shrimp  
35 farming in degraded saline areas holds the potential to bury carbon (C) in the sediments.  
36 However, studies on the mechanisms of sediment C dynamics and C-flux in response to  
37 inland saline aquaculture management practices are still scarce. Therefore, the objectives of  
38 the present study are to quantify the C burial rate in inland saline aquaculture ponds and  
39 assess the impact of inland saline aquaculture on sensitive C fractions in the bottom sediment  
40 of the ponds.

41 **Materials and methods:** The sediment samples (n = 12 from each pond) were collected  
42 from six shrimp farming ponds (1000 m<sup>2</sup> area of each pond) of different ages. The sediment  
43 depth, sediment accumulation rate and the levels of total carbon (TC), total organic carbon  
44 (TOC) and sediment oxidizable organic carbon (SOC) and its different fractions were  
45 determined using standard procedures. The data were analysed by one-way analysis of  
46 variance (ANOVA), followed by the Duncan's multiple range test for comparing the means,  
47 and the Pearson correlation test was used to assess the relationship between the different  
48 pond sediment parameters and SOC content.

49 **Results and discussion:** The results revealed that the annual C accumulation rates varied  
50 from 902 to 1346 kg C ha<sup>-1</sup> year<sup>-1</sup> in 7-year-old earthen ponds (EPs) and bottom cemented  
51 ponds (BCPs), respectively. The sediment C fractions, including TC, TOC, SOC and its  
52 fractions (very labile, VLc; labile, Lc; less labile, LLc), and non-labile carbon (NLc)) were  
53 progressively increased over the pond age. The inland saline aquaculture practices over the  
54 years increased both active (AC) and passive carbon (PC) pools in the pond sediments,  
55 helped in the restoration and improvement of sediment quality and enhanced C sequestration  
56 potential of the sediments. Furthermore, a significant increase in the level of particulate

57 organic carbon (POC) in BCPs justified that the non-ploughing practices at BCPs facilitated  
58 the formation of macro- and micro-aggregates, thereby increasing the C retention and  
59 stability of the pond sediments.

60 **Conclusion:** This study suggested that the shrimp farming ponds in semi-arid saline soils  
61 represented considerable C burial hotspots, enhanced the stable passive C pools and  
62 improved the sediment quality.

63

64 **Keywords:** Carbon accumulation, sensitive C fractions, Particulate organic carbon, Shrimp  
65 farming, aggregate formation, Active and Passive C pools.

66

## 67 **1. Introduction**

68 Soil salinization is a major land degradation problem affecting crop productivity worldwide  
69 (Moreira et al. 2020). Globally, saline soils cover an area of 954 million hectares (Mha) and  
70 account for 7-9% of agricultural productivity loss (Meena et al. 2019; Mukhopadhyay et al.  
71 2020). In India, a total 6.73 Mha area is affected by soil salinization, comprising 3.77 and  
72 2.96 Mha sodic and saline soils, respectively (Sharma et al. 2020).

73 Inland saline aquaculture is widely practiced to utilize degraded saline soils and saline  
74 groundwater in the USA, Israel, India and Australia for generating income through enhanced  
75 production of euryhaline, shrimp and marine fish species with high growth potential (Allan  
76 et al. 2009; Singha et al. 2020). Inland saline aquaculture ponds cover 650 ha area in India,  
77 and is projected to be increased further in the near future.

78 Freshwater and brackish water aquaculture ponds together cover 0.79 Mha in India, and  
79 have an enormous potential to bury carbon (C) in sediments (Adhikari et al. 2012, 2019).  
80 Globally, aquaculture ponds cover an area of 11.1 Mha and accumulate an estimated 16.6  
81 million tons C per year ( $\text{MT yr}^{-1}$ ) (Boyd et al. 2010). Similarly, the C accumulation rate in

82 0.79 Mha of aquaculture ponds of India could vary from 0.6 to 1.2 tera gram (Tg) C yr<sup>-1</sup>  
83 (Adhikari et al. 2012). Although similar cultural practices are followed in inland saline  
84 aquaculture (shrimp farming) over a wide range of areas in the world, the saline sediments  
85 pose different physico-chemical and biological properties due to different geological origins  
86 of the sediments and climato-ecological conditions (Partridge 2008; Raul et al. 2021). These  
87 saline sediments differ in their physico-chemical and biological properties, including higher  
88 sediment electrical conductivity (EC), higher bulk density and higher porosity, less SOC,  
89 poor nutrient content and availability, and pose higher salt-stress on sediment  
90 microorganisms and enzyme activities (Mukhopadhyay et al. 2020; Basak et al. 2021; Raul  
91 et al. 2021). Therefore, quantification of the rate of sediment C accumulation in inland saline  
92 shrimp farming ponds is much needed.

93 In addition, the proportion of different bottom sediment C fractions evolving in response to  
94 land-use changes could be applied as a useful tool to identify desired management practices  
95 that potentially protect C stock in the soil. Thus, the quantification of shrimp aquaculture  
96 pond sediment oxidizable organic carbon (SOC) pool is essential to understand the changes  
97 in the sediment C dynamics and C-fluxes from the shrimp aquaculture ponds. Depending on  
98 the residence time, the soil organic carbon is categorized into active (AC) and passive carbon  
99 (PC) pools (Chan et al. 2001). The AC pool constitutes the easily oxidizable C with a  
100 residence time of less than five years, while the fractions of higher residence time than AC  
101 are categorized as PC pools (Ramesh et al. 2019). Additionally, some of the critical labile  
102 pools such as particulate organic carbon (POC), permanganate oxidizable carbon (KMnO<sub>4</sub>-  
103 C), microbial biomass carbon (MBC) are widely used as the most sensitive indicators to  
104 assess the effect of different agricultural management practices on the levels of soil organic  
105 carbon and soil quality (Chen et al. 2016a; Duval et al. 2018; Thangavel et al. 2018).

106 Among all the C fractions, POC is the most sensitive C fractions for assessing the  
107 management-induced changes in the levels of soil organic carbon, and poses a significant  
108 relationship with soil physical properties and agronomic productivity (Duval et al. 2018).  
109 However, till date no report is available that has assessed the impact of aquaculture  
110 management practices on the levels of SOC and sediment quality. Thus, an understanding  
111 on the changes in the labile C fractions in pond sediments in response to aquaculture  
112 management practices are important to address the concern about maintaining and restoring  
113 sediment quality and sustainable shrimp biomass production.

114 The carbon accumulation rates in different aquaculture ponds with different cultural  
115 practices have been quantified (Adhikari et al. 2012, 2019; Kunlapapuk et al. 2019), but  
116 options for different inland saline aquaculture farming systems have not been studied yet.  
117 Moreover, mechanisms and understanding on the changes of labile sediment C fractions in  
118 response to other management practices such as ploughing, liming, manuring and feeding in  
119 inland saline aquaculture systems are required for maintaining and restoring bottom  
120 sediment organic matter (SOM) to achieve good quality sediment. Keeping the above views,  
121 the present study was formulated to: (a) quantify C burial rate in inland saline aquaculture  
122 ponds, and (b) assess the impact of inland saline aquaculture on sensitive C fractions in the  
123 bottom sediment of the ponds.

124

## 125 **2. Materials and methods**

### 126 *2.1 Study area and shrimp pond systems*

127 The study area comprised of six inland saline shrimp farming ponds in Rohtak, Haryana,  
128 India (28.8618° N, 76.4747° E). These ponds were managed by the ICAR - Central Institute  
129 of Fisheries Education, Rohtak, Haryana, India. The Pacific White shrimps (*Litopenaeus*  
130 *vannamei*) were cultured in these ponds for 5-8 years. The mean annual temperature of the

131 region is 31.8°C with an average rainfall of 597 mm, and much of the rain received during  
132 June to September. The experimental pond sediments (0-15 cm depth) were sandy loam in  
133 texture. Among six sampling ponds three earthen ponds (EPs) and three bottom cemented  
134 ponds (BCPs), having a 1000 m<sup>2</sup> area (50×20 m<sup>2</sup>) were selected for the study. The EPs were  
135 excavated earthen ponds and completely constructed from sediment materials, whereas  
136 BCPs were earthen type ponds with bottom layer lined with a layer of cement (10-15 cm  
137 thick) and a layer of sediment (10-15 cm) to control the seepage loss of water.

## 138 2.2 Shrimp pond management

139 The sediment cores were collected from six shrimp farming ponds of known age. The pond  
140 descriptions are listed in Table 1. Since the optimal requirement of temperature for shrimp  
141 (*Litopenaeus vannamei*) culture is 22 to 35°C, shrimp production in these ponds took place  
142 between July to October (3-4 months), with only a single production cycle per annum. A  
143 water level of 1.5-2.0 m was maintained in the ponds for 3-4 months during the shrimp  
144 production (July – October), and then 90% water of the ponds was drained up during harvest  
145 (November-December). The remaining 10% water was drained before the onset of the new  
146 culture cycle (May). Overall, the pond sediments remained completely dry for only one  
147 month during May just before the onset of the new culture cycle.

148 With the start of the new production cycle, the ponds were drained and dried for one month  
149 following the standard practices to remove pathogenic organisms and obnoxious gases  
150 (Kumar et al. 2013; Abraham et al. 2020). Additionally, ploughing was carried out at EPs  
151 after drying the ponds, and then water was filled by pumping saline groundwater in all the  
152 ponds up to a height of 1.5- 2.0 m. An organic fertilizer (fermented rice bran (*Oryza sativa*)),  
153 was prepared by mixing rice bran, yeast and water overnight (Adhikari et al. 2012), and  
154 applied at the rate of 250 kg ha<sup>-1</sup> to all the ponds one week before releasing the shrimp seeds

155 into the ponds. The shrimp seeds were stocked at the rate of 35-40 numbers m<sup>-2</sup> at 05:00-  
156 07:00 AM after acclimatizing for 30 min in pond water. Shrimps were fed with artificial  
157 feeds containing a minimum level of 35% crude protein and 35-40% C (on a dry weight  
158 basis). Feeding was carried out by applying three different feeds, i.e., starter feed, grower I,  
159 and grower II feeds of having different pellet sizes and shapes (Table S1). The feeds were  
160 provided based on the total expected biomass weight of the shrimps at each pond. The  
161 timelines of feeding were 6:00 AM, 10:00 AM, 2:00 PM, and 6:00 PM during each day. In  
162 each pond, one 1500 W paddlewheel aerators were operated twice a day, 4:00-8:00 AM and  
163 18:00-22:00 PM, to maintain the dissolved oxygen levels at above 5 ppm in the water. The  
164 water (pH = 8.37±0.1) chlorophyll-a concentration was estimated routinely using a UV-  
165 visible spectrophotometer (Hack DR6000™, USA) following the method of (Suzuki and  
166 Ishimaru 1990), and the average values are listed in Table 1.

### 167 *2.3 Collection and analysis of sediment samples*

168 Four sampling sites in each pond (total 6 ponds) were selected based on the adopted feeding  
169 locations and aerator's positions (Fig. 1). From each sampling site, three intact sediment  
170 cores were collected manually (total 12 samples from each pond) using a metallic core of  
171 7.5 cm inner diameter and 15 cm length, according to the methodology described by Steeby  
172 et al. (2004). The sediment depth of EPs was determined using the method suggested by  
173 (Steeby et al. 2004). The transparent plastic core sampler was slowly inserted into the pond  
174 sediment until the marked resistance indicated that contact with the original compact soil  
175 was made. Once the sediment core was collected, the sediment depth was determined by  
176 measuring the point of top layer of sediment to the point on the lower boundary, where  
177 lighter parent pond sediment could be distinguished from the darker accumulated sediment.  
178 Later on, all 12 sediment cores collected from each pond were mixed to make a single

179 composite sample representing individual pond, and were stored temporarily at 4°C. A  
180 portion of the core samples was oven-dried at 105°C for 48-72 h, and the sediment dry bulk  
181 density (BD) was computed and expressed as  $\text{Mg m}^{-3}$  (Kadam et al. 2005). A part of the air-  
182 dried, pulverized and sieved sediment sample was analysed for SOC using the dichromate  
183 oxidation technique by rapid titration method (Walkley and Black 1934). The rate of C  
184 accumulation in the pond sediment was estimated using Eq. 1 (Boyd et al. 2010; Adhikari  
185 et al. 2012, 2019):

$$186 \text{ Rate of C accumulation in sediment} = [\text{sediment BD} \times \text{sediment accumulation rate} \times \text{SOC} \\ 187 (\%)] \quad (\text{Eq. 1})$$

188 The relevant sediment physicochemical parameters were analysed using the standard  
189 American Society for Testing Materials (ASTM D-2216-05, 2008) (Table S2). Triplicate  
190 sub-samples were analysed from each of the composite sediment samples. Total carbon (TC)  
191 and total nitrogen (TN) levels ( $\text{g kg}^{-1}$ ) in the sediment samples were analysed by dry  
192 combustion method on a CHNS elemental analyser (Perkin Elmer, 2400 Series II CHNS/O,  
193 USA) (Nelson and Sommers 1996). Total organic carbon (TOC) was analysed by acid  
194 fumigation (Harris et al. 2001) followed by dry combustion method using the CHNS  
195 elemental analyser (Perkin Elmer, 2400 Series II CHNS/O, USA) (Nelson and Sommers  
196 1996). Different fractions of SOC comprising of very labile (VLc), labile (Lc), less-labile  
197 (LLc) and non-labile (NLc) carbon were determined using sulphuric acid ( $\text{H}_2\text{SO}_4$ ) aqueous  
198 solution ratios of 0.5:1, 1:1, and 2:1 (corresponding to 12 N, 18 N, and 24 N  $\text{H}_2\text{SO}_4$ ) (Chan  
199 et al. 2001). The VLc fraction was determined by oxidizing with 12 N  $\text{H}_2\text{SO}_4$ ; Lc fraction  
200 was determined by calculating the difference between SOC extracted between 18 N and 12  
201 N  $\text{H}_2\text{SO}_4$ ; LLc fraction was determined by calculating the difference between SOC extracted  
202 between 24 N and 18 N  $\text{H}_2\text{SO}_4$ ; NLc fraction was calculated from the difference between

203 TOC and SOC oxidized with 24 N H<sub>2</sub>SO<sub>4</sub>. Among the SOC fractions, VLc and Lc are AC  
204 pools, while LLc and NLc are PC pools. The POC level was determined by calculating the  
205 difference between the SOC of the whole sediment and SOC of particles that passed through  
206 53 µm sieve (Cambardella and Elliott 1992).

#### 207 *2.4 Statistical analysis of data*

208 The experimental design was a completely randomized design (CRD) with a total sample  
209 size of 12 (n=12) from each pond. The SPSS version 19.0 (SPSS Inc., USA) analytical  
210 software package was used for all the statistical analysis of data. The data were analyzed by  
211 one-way analysis of variance (ANOVA), followed by Duncan multiple range test at a 5%  
212 level of significance for comparing the means. Pearson correlation was used to assess the  
213 relationship between the different pond sediment parameters and sediment oxidizable  
214 organic carbon (SOC), and significant correlations were identified at 95% and 99%  
215 confidence level of intervals. The results were presented as mean ± standard error, and all  
216 the statistical plots were generated using Origin Pro 8.5 software package (OriginLab Corp.  
217 USA).

218

### 219 **3. Results and discussion**

#### 220 *3.1 Role of shrimp pond types on sediment carbon contents*

221 The sediment depth varied from 9.05 ± 0.8 cm in 5 year old EP to 13.6 ± 1.4 cm in 8 year  
222 old BCP (Table 2). The ponds were drained and dried before starting a new production cycle;  
223 thereby, a considerable amount of sediment was lost through runoff (Boyd et al. 2010;  
224 Adhikari et al. 2012). The sediment depth was strongly correlated with the pond age (r =  
225 0.96, *p* < 0.01). The sediment accumulation rate in these ponds varied from 1.57 to 1.87 cm

226 year<sup>-1</sup> (Table 2), and it declined with the pond age ( $r = -0.76, p < 0.05$ ). Boyd et al. (2010)  
227 and Steeby et al. (2004) also observed a decline in sediment accumulation rate as the  
228 aquaculture age increased for ponds in Thailand and Mississippi (USA), respectively. The  
229 sediment accumulation occurred due to accumulation of unconsumed feeds,  
230 fertilizers/organic manures, algae and algal-related organic matter at the pond sediment  
231 (Flickinger et al. 2020; Türk Çulha and Karaduman 2020; Junior et al. 2021). In the present  
232 study, the higher input of fertilizers (25 kg pond<sup>-1</sup> year<sup>-1</sup>) and feed (220 kg pond<sup>-1</sup> year<sup>-1</sup>)  
233 could be the major reason for the higher sediment accumulation in these ponds. Moreover,  
234 the higher sediment accumulation rate at younger aged ponds might be associated with  
235 higher levees erosion at the younger ponds (Steeby et al. 2004). This could also be related  
236 to the increased primary production when considering the higher chlorophyll-a  
237 concentration (Table 1) in 5 years old ponds than 8 years old earthen ponds (Ramos e Silva  
238 et al. 2017; Junior et al. 2021).

239 The BD of the pond sediments ranged from 0.97 to 1.14 g cm<sup>-3</sup>, and was negatively  
240 correlated with pond age ( $r = -0.58, p < 0.05$ ) and sediment depth ( $r = -0.73, p < 0.01$ ) (Table  
241 3). In addition, BD also showed a significant negative correlation with SOC concentration  
242 ( $r = -0.77, p < 0.01$ ) (Table 3). The negative relationship between BD and SOC concentration  
243 was mainly attributed to the likely conversion of some micropores into large macropores  
244 due to the cementing action of polysaccharides and organic acids formed during the  
245 decomposition of SOM (Brar et al. 2013). The SOC concentration gradually increased over  
246 the years of the culture period, with the lowest concentration of 0.48% in 5 years old EP to  
247 0.80% in 7 years old BCP (Table 2). The observed SOC concentrations were lower than the  
248 values reported by Adhikari et al. (2019) in the sediments of freshwater aquaculture ponds  
249 of Andhra Pradesh, India. However, the values were similar to values reported by Anikuttan  
250 et al. (2016) (0.32-0.91%) in the sediments of freshwater aquaculture ponds of Orissa, India.

251 Noteworthy that the SOC concentrations in aquaculture ponds may vary on several factors  
252 including pond age, primary productivity, climato-ecological and hydrological conditions,  
253 and aquaculture practices (Boyd et al. 2010; Adhikari et al. 2012; Chen et al. 2016b;  
254 Flickinger et al. 2020; Junior et al. 2021).

255 The carbon accumulation rate in the pond sediment varied from 902 kg C ha<sup>-1</sup> year<sup>-1</sup> in 7  
256 years old EP to 1346 kg C ha<sup>-1</sup> year<sup>-1</sup> in 7 years old BCP, respectively (Table 2). The carbon  
257 accumulation rate at BCPs was 7% and 49% higher than EPs of 5 years and 7 years old  
258 ponds, respectively. Our results corroborated with the previous results in shrimp culture  
259 ponds (1099 ± 75 kg C ha<sup>-1</sup> year<sup>-1</sup>) of Odisha, India (Adhikari et al. 2012). Similarly, Boyd  
260 et al. (2010) observed the similar values in 11 years old shrimp ponds (940 kg C ha<sup>-1</sup> year<sup>-1</sup>)  
261 at Choluteca, Honduras. However, our observed values were much lower than those reported  
262 by Boyd et al. (2010) in 5 years old shrimp ponds (2740 kg C ha<sup>-1</sup> year<sup>-1</sup>) of Khao Chakan,  
263 Thailand. The feed and fertilizer inputs, and photosynthetic activity (chlorophyll-a, (Table  
264 1)) of phytoplankton might have contributed to the accumulation of SOC in the pond bottom  
265 sediment (Chen et al. 2016b; Flickinger et al. 2020). The lower C: N ratio (4.47-6.84) and  
266 higher N content (5.64-7.78%) of the shrimp feeds (Table S1) and available C source from  
267 the organic wastes could have increased the autotrophic and heterotrophic activities at the  
268 pond sediment (Junior et al. 2021; Xu et al. 2016) and resulted in the formation of stable  
269 SOC in the pond sediments. The results indicated that the inland saline shrimp farming  
270 ponds accumulated a comparable amount of C to the previous reported figures for freshwater  
271 and brackish water ponds of India (Adhikari et al. 2012, 2019). Thus, the present study  
272 revealed that the conversion of degraded saline soils into shrimp farming ponds could help  
273 to accumulate a considerable amount of C over an extended period.

274

275 *3.2 Effect of aquaculture practices on carbon stock*

276 At 0-10 cm sediment depth, the sediment TC levels varied from 8.7 to 12.96 g kg<sup>-1</sup> of  
277 sediment (Fig.2). The TC levels were 2%, 24%, and 18% higher at 5 years, 7 years, and 8  
278 years old BCPs than EPs, respectively. Among all the ponds, P2 had the most elevated TC  
279 levels, whereas P5 had the lowest TC concentration. The TOC levels in aquaculture ponds  
280 ranged from 8.17 to 12.04 g kg<sup>-1</sup> of sediment, accounting for 87 - 98% of the TC stock. The  
281 TOC levels in 5, 7, and 8 years BCPs were observed to be relatively higher than respective  
282 EPs; however, significantly higher TOC was observed in 7 years old BCP than same age  
283 EP. In comparison to the Eps, the BCPs had higher TOC in 5 years and 8 years old ponds  
284 likely due to ploughing which significantly affected the distribution and stabilization of  
285 SOC, thereby depleted the TOC over a long period of aquaculture practices (Briedis et al.  
286 2012; Bongiorno et al. 2019). The TIC accounted for 2.5-12.5% of the sediment TC levels  
287 across the ponds.

288 The feeding and fertilization of the ponds resulted in higher CO<sub>2</sub> fixation by phytoplankton  
289 and increased the TOC and TIC levels of the pond sediment (Chen et al. 2016b; Flickinger  
290 et al. 2020; Junior et al. 2021). Anikuttan et al. (2016) reported a lower TOC concentration  
291 at unutilized aquaculture ponds than regularly used aquaculture ponds. Flickinger et al.  
292 (2020) observed a strong positive correlation between chlorophyll-a content and total  
293 suspended solids during sedimentation, and suggested that the primary production was a key  
294 process in the aquaculture ponds by absorbing CO<sub>2</sub> from atmospheric and autochthonous  
295 sources leading to an increase in TOC levels in the pond sediments. Compared to EPs, the  
296 BCPs had 6.1 to 39.7% higher TOC concentration in the pond sediments. The management  
297 practices in EPs involved regular ploughing of the pond bottom, which disturbed the  
298 distribution and stabilization of sediment aggregates, and exposed SOC to rapid microbial  
299 decomposition and subsequently depleted the TOC (Plaza-Bonilla et al. 2014; Prasad et al.  
300 2016). In the present study, the TC and TOC levels were found to progressively increase

301 with pond age (Fig 2), indicating that the aquaculture practice enhanced the C stock in the  
302 pond sediments through sediment carbon accumulation. The pond sediment had a pH value  
303 around  $8.2 \pm 0.4$  (Table S2), which indicated the possibility of carbonates and bicarbonates  
304 of  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in the pond sediments (Choudhary and Kharche 2018). This could be  
305 the reason of formation of TIC in the pond sediments over the years at the semi-arid study  
306 location in India.

### 307 *3.3 Effect of aquaculture practices on sediment oxidizable organic carbon (SOC) and its* 308 *fractions*

309 The sediment TOC was further divided into an oxidizable labile fraction and non-labile  
310 fraction to assess the impact of aquaculture management practices on SOC. The different  
311 SOC fractions significantly ( $p < 0.01$ ) varied with the chrono-sequence, and increased  
312 dramatically over the pond age (Table 4). The SOC levels varied from 4.81- 6.19  $\text{g kg}^{-1}$  in  
313 EPs to 5.17- 8.01  $\text{g kg}^{-1}$  in BCPs, accounting for 59- 67% of TOC in the pond sediments,  
314 and in all the ponds, SOC progressively increased over the pond age (Table 4). The non-  
315 labile fraction of C ranged from 3.36 to 5.31  $\text{g kg}^{-1}$  of sediment, contributing 38-44% of  
316 TOC levels of the ponds. Previous studies reported that the feed organic C input accounted  
317 for 80 to 94% of all C inputs to the SOC, and this could play a significant role in the aquatic  
318 C cycle (Flickinger et al. 2020; Zhang et al. 2018). Similarly, Flickinger et al. (2020)  
319 reported that the absorption of atmospheric  $\text{CO}_2$  by planktons in freshwater aquaculture  
320 ponds was approximately 6 to 23 times that of  $\text{CO}_2$  emitted to the atmosphere, which was  
321 pivotal source of organic C accumulation in the pond sediments (Flickinger et al., 2020;  
322 Zhang et al., 2018). High input of atmospheric  $\text{CO}_2$  and increased accumulation of C in the  
323 pond sediments suggested a rapid conversion of  $\text{CO}_2$  by phytoplankton into SOC (Flickinger  
324 et al. 2020; Zhang et al. 2020). In the current study, the TC added to each pond through  
325 shrimp feeds was 7661- 8487  $\text{kg C ha}^{-1}$  pond (Table S1), which could be the major source

326 of SOC into these aquaculture ponds. Also, the feed input provided major nutrients to  
327 microorganisms and facilitated the higher atmospheric CO<sub>2</sub> fixation at the pond sediments  
328 (Silva et al. 2017; Junior et al. 2021). The rapid conversion of CO<sub>2</sub> by phytoplankton could  
329 be observed from the trend of the chlorophyll-a data analysed in the pond water (Table 1),  
330 and this could also justify the increased SOC in the pond sediment. Furthermore, the higher  
331 bioturbation produced by the shrimps might have exposed more buried organic C to aerobic  
332 mineralization and liberation of nutrients to the water column, increasing the photosynthesis  
333 and fixation of C at the pond bottom sediments (Green and Boyd 1995; Joyni et al. 2011;  
334 Flickinger et al. 2020).

335 In the sediment samples, the relative magnitude of the different SOC fractions followed the  
336 trend: VLc > Lc > LLc, which respectively comprised of about 43-54%, 31-41% and 9-22%  
337 of SOC. The increased levels of NLc over the years of shrimp culture was likely due to the  
338 non-degradable refractory fractions of algae, algal organic matter, including non-  
339 hydrolysable biopolymers (algeanans) (Marin-Batista et al. 2020.; Ras et al. 2011) and  
340 antibacterial chlorophyll-derived compounds (e.g., chlorophyllides) (Jewell and McCarty  
341 1971).

342 Of TOC, active pools (VLc + Lc) constituted 47- 56% of TOC, and were prone to be lost  
343 easily (Fig. 3). The PC pools (LLc + NLc) accounted for 40-49% of sediment TC, and 44-  
344 53% of TOC of pond sediments (Fig 3, Table S3). In BCPs, the PC pools accounted for 48-  
345 53% of sediment TOC levels, whereas it accounted for 44-50% of sediment TOC in EPs.  
346 The quantity and quality of added organic matter and the nutrient availability in the ponds  
347 governed the concentration of different C pools and C accumulation patterns (Bhardwaj et  
348 al. 2019). The relative proportions of AC and PC were dependent upon the availability of  
349 nutrients (Nath et al. 2018), and a large portion of passive pools indicated the relative  
350 stability of the organic C stock in the system (Sarkar et al. 2015). Since PC pools are less

351 prone to oxidation than AC pools (Sarkar et al. 2015; Nath et al. 2018), the high proportion  
352 of PC pools in BCPs indicated the relative stability of SOC in BCPs compared to EPs. Thus,  
353 the inland saline aquaculture in degraded saline soils increased both AC and PC pools in the  
354 pond sediments, helped in the restoration and improvement of sediment quality, and  
355 enhanced the retention and stability of C in the pond sediments.

356

### 357 *3.4 Effect of aquaculture practices on particulate organic carbon (POC)*

358 The POC is mainly composed of decomposing plant, animal and microbial residues (Feller  
359 and Beare 1997; Yan et al. 2007; Mi et al. 2016). The POC differed statistically between all  
360 the aquaculture ponds, and ranged from 0.29 to 4.09 g kg<sup>-1</sup> (Fig. 3, Table S3). Here, we  
361 observed an increasing trend in the concentration of POC from 0.29 to 1.024 g kg<sup>-1</sup> of  
362 sediment in EPs, and the mean POC concentration in BCPs varied from 1.32- 4.09 g kg<sup>-1</sup>,  
363 accounting for 15 to 34% of the TOC levels. The dominant chemical constituents of POC  
364 includes phenol, hemicellulose, and microbial and fungal derived xylanase and chitin  
365 (Lavallee et al. 2020). The low C: N ratio (4.47-6.84) of shrimp feeds might have facilitated  
366 the faster decomposition of SOM, and increased the levels of phenol, hemicellulos,  
367 microbial and fungal residues in the pond sediments, thereby facilitating the macro- and  
368 micro-aggregate formation (as POC) over time (Mi et al. 2016) . The low level of POC in  
369 EPs might be attributable to the intensive management practices employed to the pond  
370 sediments, i.e., ploughing once in a year. Ploughing might disrupt both macro- and  
371 microaggregates, increase the sediment temperature and aeration, facilitating the release of  
372 C from SOM which was otherwise protected in sediment aggregates (Six et al. 1999;  
373 Bongiorno et al. 2019). Ploughing could facilitate the incorporation of organic matter into  
374 soils, favouring the mineralization of POC by soil microorganisms (Bongiorno et al. 2019;  
375 Kan et al. 2020; He et al. 2021). The input of allochthonous organic debris from bottom

376 macrophytes might have reinforced the development and stabilization of micro-aggregates  
377 within macro-aggregates of the sediments that would help to protect POC from rapid  
378 decomposition in BCPs (Thangavel et al. 2018; Bongiorno et al. 2019; Kan et al. 2020). The  
379 SOM added through allochthonous and autochthonous sources could have experienced  
380 greater physico-chemical transformation, and then were stabilized in the aggregates through  
381 binding on to the mineral surfaces and became biochemically recalcitrant (Krull et al. 2003;  
382 Ramesh et al. 2019) in the pond sediments over many years of cultural practices.

383

384

#### 385 **4. Conclusions**

386 This study was focussed to evaluate the sediment C accumulation potential and sensitive  
387 sediment C fractions of inland saline shrimp farming ponds. The sediment accumulation rate  
388 and SOC increased over the years of culture practices with much higher levels in BCPs than  
389 EPs. Overall, the SOC accumulation rates in these inland saline shrimp farming ponds  
390 ranged from 902 to 1346 kg C ha<sup>-1</sup> yr<sup>-1</sup> with maximum accumulation potential was observed  
391 in BCPs compared to EPs. Nonetheless, both AC and PC pools increased with pond age,  
392 with PC pools were significantly higher in non-ploughed BCPs than EPs. The evaluation of  
393 POC revealed that the ploughing practices in EPs disrupted the macro- and micro-aggregates  
394 and could have accelerated the decomposition of labile C pools which resulted in lower TOC  
395 in EPs. Therefore, the inland saline shrimp farming ponds could act as critical C burial  
396 hotspots in semi-arid areas of the world, and over the years of culture, could increase the C  
397 stock of the systems, enabling SOC restoration. Further investigations are needed to assess  
398 the impact of ploughing, manuring, and feeding practices on changes in the sensitive C  
399 fractions and C management indices in the saline aquaculture pond systems.

400

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405 **Availability of data and material**

406 The data that support the findings of the study are available from the corresponding author  
407 upon reasonable request.

408

409 **Conflict of interest**

410 The authors declare no conflict of interest.

411

412 **CRedit authorship contribution statement**

413 VKA: Experiment design, execution, sampling and analysis, Manuscript first draft  
414 preparation and revisions, VSB: Conceptualization of the research idea, Experimental  
415 design and supervision, RM: Co-supervision, Manuscript preparation, improvement and  
416 editing, SP: Execution of the experiments, Manuscript editing, VH: Execution of the  
417 experiments, GRB: Manuscript editing, GT: Conceptualization of the research idea, GK:  
418 Conceptualization of the research idea, BS: Data interpretation, Manuscript preparation,  
419 improvement and editing.

420

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- 606

607 **Title of tables**

608 **Table 1.** Characteristics of different aquaculture ponds and their corresponding management  
609 practices

610 **Table 2.** Carbon accumulation rates in sediments from different shrimp farming ponds

611 **Table 3.** Pearson correlations between pond sediment parameters and sediment oxidizable  
612 organic carbon (SOC)

613 **Table 4.** Changes in the level of SOC and its fractions ( $\text{g kg}^{-1}$ ) over the years of shrimp  
614 farming practices

615 **Legend of figures**

616 **Figure 1.** Location of the different sampling sites in the inland saline shrimp farming ponds  
617 (P1- Pond 1, P2- Pond 2, P3- Pond 3, P4-Pond 4, P5- Pond 5, P6- Pond 6; S1-Sampling Site  
618 1, S2-Sampling Site 2, S3- Sampling Site 3, S4- Sampling Site 4; Total sample size  $n=12$  from  
619 each pond).

620 **Figure 2.** Effect of shrimp farming management practices on sediment C fractions ( $\text{g kg}^{-1}$ ) ( $n$   
621  $= 12$ ,  $p < 0.05$ ) of different shrimp aquaculture ponds.

622 **Figure 3.** Effect of shrimp farming on active C-fractions (AC), passive C- fractions (PC) and  
623 particulate organic carbon (POC) ( $\text{g kg}^{-1}$ ) ( $n = 12$ ,  $p < 0.05$ ) in shrimp aquaculture pond  
624 sediments.

625

626 **Tables**

627 **Table 1.** Characteristics of different aquaculture ponds and their corresponding management practices

628

Pond	Pond type	Age	Initial chlorophyll-a content (mg L <sup>-1</sup> )	Final chlorophyll-a content (mg L <sup>-1</sup> )	Management practices
P1	Earthen pond	8	0.08	1.08	Ploughing, draining, drying, fertilization and feeding.
P2	Bottom cemented earthen pond	8	0.06	1.02	No ploughing, draining, drying, fertilization and feeding.
P3	Bottom cemented earthen pond	7	0.05	1.02	No ploughing, drying, draining, fertilization, and feeding.
P4	Earthen pond	7	0.08	1.03	Ploughing, drying, draining, fertilization and feeding.
P5	Earthen pond	5	0.14	1.20	Ploughing, draining, drying, fertilization and feeding.
P6	Bottom cemented earthen pond	5	0.10	1.18	No ploughing, drying, fertilization and feeding.

629

630

631 **Table 2.** Carbon accumulation rates in sediments from different shrimp farming ponds

632

Pond	Age	Sediment depth (cm)	Sediment accumulation rate (cm yr <sup>-1</sup> )	Sediment dry bulk-density (g cm <sup>-3</sup> )	Sediment oxidizable organic carbon (%)	<b>Carbon accumulation rate in sediment (kg ha<sup>-1</sup> yr<sup>-1</sup>)</b>
P1	8	12.9 ± 1.2 <sup>¶</sup>	1.62 ± 0.02	1.07	0.62 <sup>b</sup> ± 0.04	1073 <sup>b</sup> ± 75
P2	8	13.6 ± 1.4	1.71 ± 0.01	0.97	0.64 <sup>b</sup> ± 0.01	1054 <sup>b</sup> ± 30
P3	7	11.6 ± 1.2	1.66 ± 0.02	1.01	0.80 <sup>c</sup> ± 0.01	1346 <sup>c</sup> ± 28
P4	7	10.8 ± 1.4	1.57 ± 0.03	1.14	0.50 <sup>a</sup> ± 0.02	902 <sup>a</sup> ± 28
P5	5	9.05 ± 0.8	1.81 ± 0.03	1.13	0.48 <sup>a</sup> ± 0.015	987 <sup>ab</sup> ± 52
P6	5	9.35 ± 0.5	1.87 ± 0.05	1.09	0.52 <sup>a</sup> ± 0.01	1055 <sup>a</sup> ± 54

633

634 <sup>¶</sup>Values within a column followed by different letters are significantly different at  $p < 0.05$ , as obtained from Duncan multiple range test.

635

636 **Table 3.** Pearson correlations between pond sediment parameters and sediment oxidizable organic carbon (SOC)

	Age of the pond	Sediment depth	Sediment accumulation rate	Dry bulk-density	Sediment oxidizable organic carbon (%)
Age of the pond	1	0.96**	-0.76**	-0.58*	NS
Sediment depth		1	NS	-0.73**	NS
Sediment accumulation rate			1	NS	NS
Dry bulk-density				1	-0.77**

637

638

\* and \*\*, significant at  $p < 0.05$  and  $p < 0.01$  respectively, NS: non-significant

639

640 **Table 4.** Changes in the level of SOC and its fractions ( $\text{g kg}^{-1}$ ) over the years of shrimp farming practices

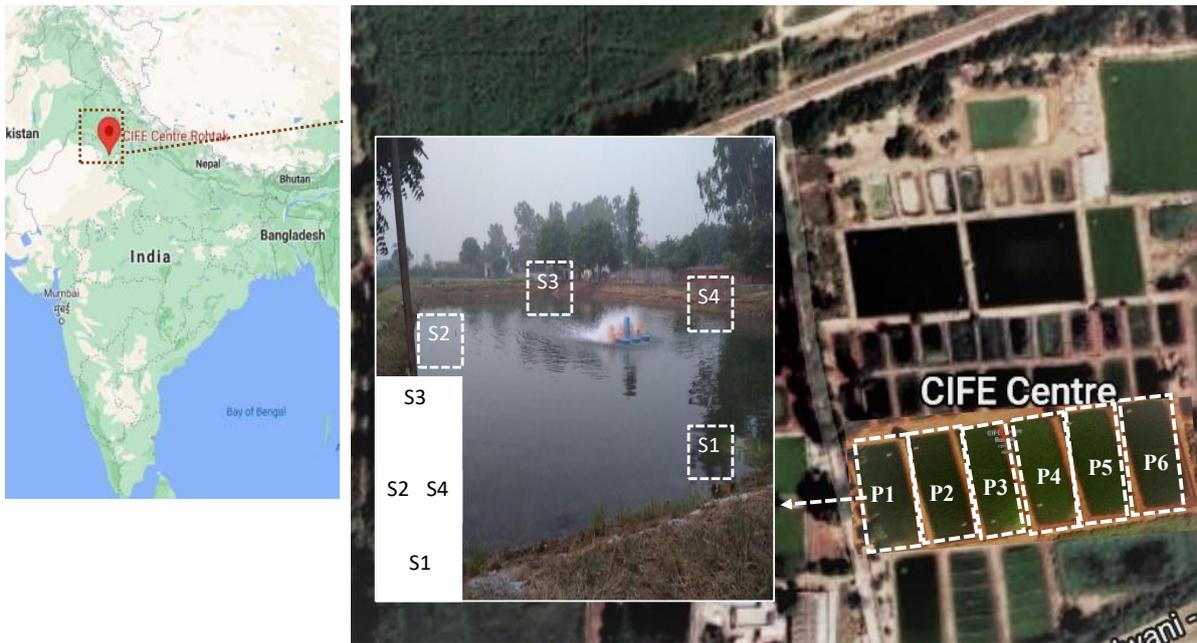
Pond	Total organic carbon	<b>Sediment oxidizable organic carbon (SOC)</b>	Very-labile carbon (VLc)	Labile carbon (Lc)	Less-labile carbon (LLc)	Non-labile carbon (NLc)
P1	9.96 <sup>ab¶</sup>	6.19 <sup>b</sup> $\pm$ 0.36	3.34 <sup>c</sup> $\pm$ 0.44	2.26 <sup>abc</sup> $\pm$ 0.51	0.58 <sup>a</sup> $\pm$ 0.2	3.77 <sup>a</sup> $\pm$ 0.36
P2	12.04 <sup>b</sup>	6.74 <sup>b</sup> $\pm$ 0.33	3.06 <sup>bc</sup> $\pm$ 0.29	2.62 <sup>bc</sup> $\pm$ 0.01	1.06 <sup>a</sup> $\pm$ 0.04	5.31 <sup>b</sup> $\pm$ 0.07
P3	11.98 <sup>b</sup>	8.01 <sup>c</sup> $\pm$ 0.145	3.42 <sup>c</sup> $\pm$ 0.07	2.84 <sup>c</sup> $\pm$ 0.22	1.75 <sup>b</sup> $\pm$ 0.15	3.98 <sup>a</sup> $\pm$ 0.14
P4	8.57 <sup>a</sup>	5.02 <sup>a</sup> $\pm$ 0.22	2.47 <sup>ab</sup> $\pm$ 0.14	1.75 <sup>ab</sup> $\pm$ 0.29	0.8 <sup>a</sup> $\pm$ 0.22	3.54 <sup>a</sup> $\pm$ 0.22
P5	8.17 <sup>a</sup>	4.81 <sup>a</sup> $\pm$ 0.15	2.18 <sup>a</sup> $\pm$ 0.15	1.97 <sup>abc</sup> $\pm$ 0.07	0.65 <sup>a</sup> $\pm$ 0.07	3.36 <sup>a</sup> $\pm$ 0.14
P6	8.67 <sup>a</sup>	5.17 <sup>a</sup> $\pm$ 0.07	2.84 <sup>abc</sup> $\pm$ 0.07	1.6 <sup>a</sup> $\pm$ 0.14	0.73 <sup>a</sup> $\pm$ 0.01	3.5 <sup>a</sup> $\pm$ 0.07

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642 <sup>¶</sup>Values within a column followed by different letters are significantly different at  $p < 0.05$ , as obtained from Duncan multiple range test.

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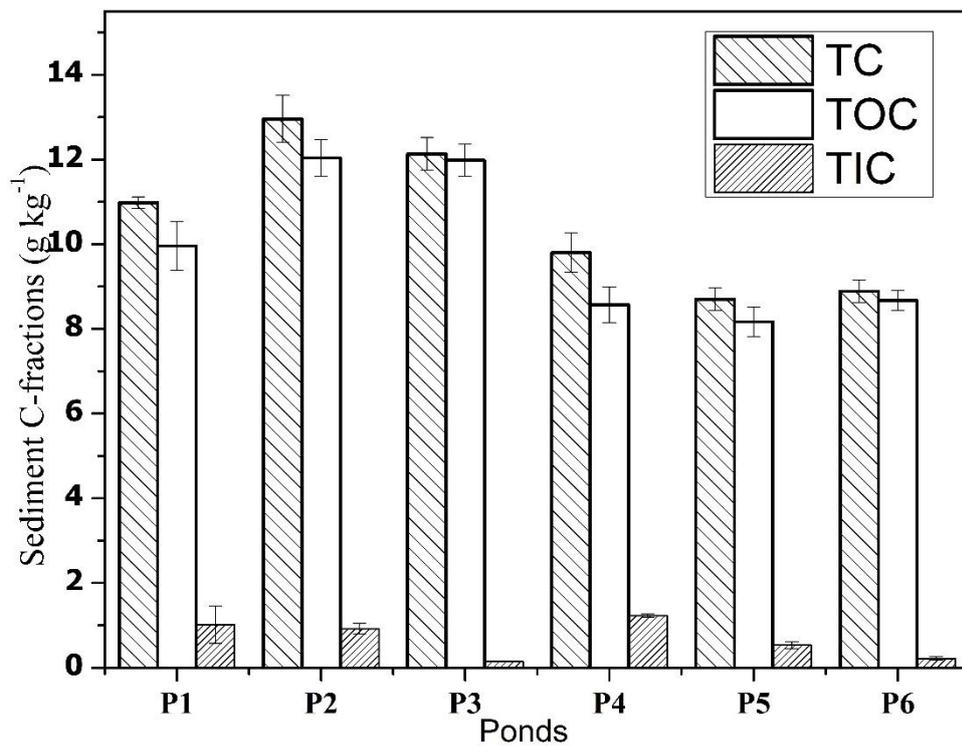
644 **Figures**



645

646 **Fig. 1.** Location of the different sampling sites in the inland saline shrimp farming ponds (P1-  
647 Pond 1, P2- Pond 2, P3- Pond 3, P4-Pond 4, P5- Pond 5, P6- Pond 6; S1-Sampling Site 1, S2-  
648 Sampling Site 2, S3- Sampling Site 3, S4- Sampling Site 4; Total sample size n=12 from each  
649 pond).

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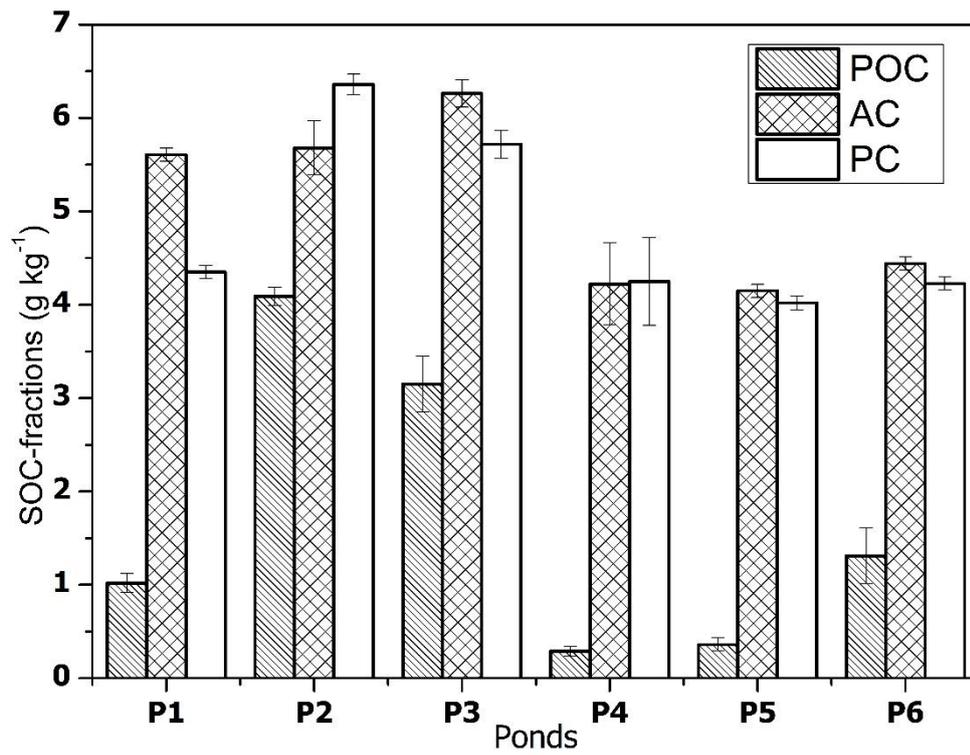


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652 **Fig 2.** Effect of shrimp farming on sediment C fractions (g kg<sup>-1</sup>) (n = 12, *p* < 0.05) of

653 different shrimp aquaculture ponds.

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656 **Fig 3.** Effect of shrimp farming on active C-fractions (AC), passive C- fractions (PC) and  
 657 particulate organic carbon (POC) (g kg<sup>-1</sup>) (n = 12, *p* < 0.05) in different shrimp aquaculture  
 658 pond sediments.

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**Supporting Information for:**

**Inland saline aquaculture increased carbon accumulation rate and stability in pond sediments under semi-arid climate**

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678 **Tables**679 **Table S1.** Classification of culture periods based on feeding strategy

Culture stage	Duration (days)	Feed type	Feed shape and size	Feeding frequency (kg ha <sup>-1</sup> )	% TC	% TN	C:N ratio
Initial	0-30	Starter feed	Crumble and 0.9-1.2 mm	40	38.58	5.64	6.84
Middle	31-60	Grower I	Pellet and 1.2 × 2-3 mm	80	34.82	7.78	4.47
Final	60-120	Grower II	Pellet and 2 × 3-4 mm	100	37.50	7.78	4.82

680 TC: total carbon; TN: total nitrogen

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682 **Table S2.** Initial physicochemical parameters of pond sediments

Sediment parameter	Value
pH	8.2 ± 0.4
EC (dS m <sup>-1</sup> )	5.37 ± 0.14
Organic carbon (%)	0.59 ± 0.02
Total carbon (%)	1.058 ± 0.35

Bulk density (g cm <sup>-3</sup> )	1.06 ± 0.09
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684 **Table S3.** Effect of 5-8 years of aquaculture practices on different C-fractions (g kg<sup>-1</sup>)

Pond	Active C pool	Passive C pool	POC
P1	5.61 <sup>b</sup> ± 0.07 <sup>¶</sup>	4.35 <sup>a</sup> ± 0.07	1.024 <sup>bc</sup> ± 0.15
P2	5.68 <sup>b</sup> ± 0.29	6.36 <sup>b</sup> ± 0.29	4.09 <sup>d</sup> ± 0.14
P3	6.26 <sup>b</sup> ± 0.14	5.72 <sup>b</sup> ± 0.14	3.15 <sup>d</sup> ± 0.29
P4	4.22 <sup>a</sup> ± 0.04	4.25 <sup>a</sup> ± 0.44	0.29 <sup>a</sup> ± 0.05
P5	4.15 <sup>a</sup> ± 0.07	4.02 <sup>a</sup> ± 0.07	0.37 <sup>ab</sup> ± 0.07
P6	4.44 <sup>a</sup> ± 0.07	4.23 <sup>a</sup> ± 0.07	1.32 <sup>c</sup> ± 0.29

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686 <sup>¶</sup>Values within a column followed by different letters are significantly different at  $p < 0.05$  as  
 687 obtained from Duncan multiple range test.

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