



# Carbon dioxide and methane fluxes across the sediment–water interface in different grass carp *Ctenopharyngodon idella* polyculture models

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**ABSTRACT:** Benthic fluxes of carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>) were evaluated in grass carp *Ctenopharyngodon idella* (G), silver carp *Hypophthalmichthys molitrix* (S), and bighead carp *Aristichthys nobilis* (B) polyculture systems, to which topmouth culter *Erythroculter ilishaeformis* (T), Pacific white shrimp *Litopenaeus vannamei* (P), or common carp *Cyprinus carpio* (C) were added. These systems, GSBT, GSBP, and GSBC, respectively, were monitored over 5 mo (May to September 2014). The presence of *L. vannamei* and *C. carpio* increased CO<sub>2</sub> and CH<sub>4</sub> emissions in GSBP and GSBC, respectively. From June to August, CO<sub>2</sub> and CH<sub>4</sub> fluxes increased in the 3 systems, and in July and August these fluxes were significantly higher in GSBC than in GSBP and GSBT, and significantly higher in GSBP than in GSBT. The metabolisms of *L. vannamei* and *C. carpio* contributed to decrease dissolved oxygen, pH, and oxidation–reduction levels, and the bioturbation of the upper sediment layers caused by these species increased organic matter degradation rate, resulting in a significant increase in particulate and dissolved organic carbon in the overlying water. Our results indicate that improving feeding efficiency and reducing organic carbon accumulation on the bottom of aquaculture ponds, as well as increasing dissolved oxygen and decreasing bioturbation across the sediment–water interface during farming seasons, is likely to reduce CO<sub>2</sub> and CH<sub>4</sub> release from aquaculture pond sediment, especially in July and August.

**KEY WORDS:** Carbon dioxide fluxes · Methane fluxes · Grass carp polyculture ponds · Sediment–water interface · Bioturbation

## INTRODUCTION

Carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>), the major atmospheric long-lived greenhouse gases (LLGHGs), are infrared absorbers and have been implicated in global warming, due to their increasing atmospheric concentrations since the industrial revolution. Whereas CO<sub>2</sub> concentration increased from 280.0 to 389.0 ppm from 1800 to 2010 (Tarasova et al. 2012), CH<sub>4</sub> increased from approximately 0.7 to 1.8 ppm from 1999 to 2005 (Forster et al. 2007). Global warming due to increasing concentrations of LLGHGs in the atmosphere is likely to affect ecosystem structure and functioning, and many studies

have focused on CO<sub>2</sub> and CH<sub>4</sub> fluxes across the water–air interface in aquatic ecosystems, including oceans, rivers, reservoirs, and lakes (Smith et al. 2000, Huttunen et al. 2002, Bange 2006, Hirota et al. 2007, Zhang et al. 2008, Tremblay & Bastien 2009, Koné et al. 2010). Although several studies have considered CO<sub>2</sub> and CH<sub>4</sub> fluxes across the sediment–water interface in these aquatic systems (Moosavi et al. 1996, Liikanen & Martikainen 2003, Adams 2005, Huttunen et al. 2006), data on freshwater aquaculture ponds are still limited.

Globally, aquaculture provides around 47% of all fish supply destined for direct human food consumption, accounting for a growing percentage of the total

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fish supply from approximately 13 % in 1990 to 40 % in 2010. About 90 % of the global aquaculture production occurs in Asia, and China alone is responsible for more than 60 % of that production (FAO 2013). China's total aquaculture production in 2014 reached 64.6 million metric tons (Mt), with freshwater aquaculture accounting for 45.51 % of this volume (Fisheries Department of Agriculture Ministry of China 2015). Freshwater aquaculture ponds are estimated to occupy 87 500 km<sup>2</sup> worldwide, 25 669 km<sup>2</sup> (29.34 %) of which are located in China (Verdegem & Bosma 2009). Freshwater aquaculture ponds sequester about 13.1 Mt yr<sup>-1</sup> of carbon globally, as organic carbon settles to the bottom of the ponds (Boyd et al. 2010, Adhikari et al. 2012). Therefore, China's freshwater aquaculture ponds play an important role in the global supply of aquatic products as well as in carbon sequestration.

The boundary between water and sediment, i.e. the sediment–water interface, shows significant variations in its physical, chemical, and biological characteristics (Schindler 1981, Nixon 1986), and plays a vital role in the circulation, transfer, and storage of materials in aquatic ecosystems (Santschi et al. 1990). Sediment is a source of LLGHGs such as CH<sub>4</sub> and CO<sub>2</sub> (Adams 2005, Beaulieu et al. 2009, Atkins et al. 2013), which are dissolved in the overlying water, or are emitted to the atmosphere through molecular diffusion, plant transport, or gas bubbles (Belger et al. 2011). Both CH<sub>4</sub> and CO<sub>2</sub> might originate from the organic carbon accumulated in freshwater pond substrates due to uneaten feed, organic fertilizers, dead plankton, and cultured species excreta (Bachoon & Jones 1992, Liikanen et al. 2002, Algsten et al. 2005, Shalini et al. 2006, Laverman et al. 2010, Adhikari et al. 2012). However, detailed studies quantifying CO<sub>2</sub> and CH<sub>4</sub> fluxes across the sediment–water interface in freshwater aquaculture ponds have not been conducted so far.

Most species produced in aquaculture systems, such as carp, feed low on the food chain in their natural habitats (FAO 2013). In China, aquaculture systems are often stocked with several species of carp, including grass carp *Ctenopharyngodon idella* (G), silver carp *Hypophthalmichthys molitrix* (S), and bighead carp *Aristichthys nobilis* (B) (Zhang et al. 2011). Grass carp is one of the most popular freshwater aquaculture species worldwide, and accounts for about 18.31 % of the total yield of freshwater aquaculture in China (Fisheries Department of Agriculture Ministry of China 2015). China's rapidly growing population and steep increase in demand for protein require the intensive utilization of in-

creasingly scarce resources, and thus innovative solutions are necessary to intensify freshwater aquaculture production to keep pace with population increase. Considering ecological and economic concerns, polyculture (i.e. the simultaneous culture of several species) has been used as a means of species diversification (Soto 2009), and numerous pelagic or benthonic organisms, such as the topmouth culter *Erythroculter ilishaeformis*, the common carp *Cyprinus carpio*, and the Pacific white shrimp *Litopenaeus vannamei*, are generally stocked in inland ponds to take full advantage of the available space (Naylor et al. 2000, Dong 2011, Zhu et al. 2011, Han et al. 2015). Ponds stocked with these species have become important polyculture models in northern China.

From an environmental perspective, the impact of new freshwater aquaculture production technologies on the emission of greenhouse gases deserves attention. Acting as bioturbators, macrobenthos can promote the degradation rate of organic matter (OM), by modifying sediment textures and changing oxygen supply due to burrowing, feeding, respiration, and excretion (Jordan & Valiela 1982, Jones et al. 1994, Levinton 1995, Welsh 2003, Kogure & Wada 2005, Meysman et al. 2006, Otani et al. 2010). These processes can also promote OM mineralization, via microbial respiration, and gas emissions in the water–sediment interface (Kikuchi 1986, Wheatcroft 2006). In the present study, *E. ilishaeformis* (T), *L. vannamei* (P), and *C. carpio* (C) were added to *C. idella* polyculture ponds to create 3 different polyculture systems, in which CO<sub>2</sub> and CH<sub>4</sub> fluxes across the water–sediment interface were evaluated. The present study aimed to test the hypothesis that stocking *C. idella* polyculture ponds with benthic species may lead to long-term increases in CO<sub>2</sub> and CH<sub>4</sub> fluxes across the sediment–water interface. In addition, the effects of *C. carpio* and *L. vannamei* on the CO<sub>2</sub> and CH<sub>4</sub> fluxes were determined and screened for differences, and the geochemical principle influencing CO<sub>2</sub> and CH<sub>4</sub> release was analyzed, considering several environmental parameters in the sediment–water interface.

## MATERIALS AND METHODS

### Experimental ponds

The present study was conducted at a freshwater fish farm in Gaoqing, Shandong Province, China (37° 04' N, 117° 33' E), which has a typical temperate monsoonal climate, with a mean annual temperature of 13.1°C. Nine representative *Ctenopharyngodon*

*idella* polyculture ponds, with an area of 0.25 hm<sup>2</sup> and an average water depth of 1.8 ± 0.5 m, were selected and 3 polyculture systems were investigated: (1) GSBT: *C. idella*, *Hypophthalmichthys molitrix*, *Aristichthys nobilis*, and *Erythroculter ilishaeformis*; (2) GSBP: *C. idella*, *H. molitrix*, *A. nobilis*, and *Litopenaeus vannamei*; and (3) GSBC: *C. idella*, *H. molitrix*, *A. nobilis*, and *Cyprinus carpio*. Three replicates were established for each treatment. Pellet feed was provided every day at 07:00, 10:00, 13:00, and 16:00 h, and pond water was not changed during the experiment. The basic details and starting conditions of the surface sediment in the 3 systems are given in Tables 1 & 2, respectively.

### Sampling

Bottom-water and sediment samples were collected from 3 sampling sites within each pond, around the 15th day of every month, from May to September 2014. The 3 sampling sites were evenly positioned on a circle centered on the aerator. The radius of the circle was 10–11 m and the distance between sampling sites was 17–18 m. In addition, the diel variation in CO<sub>2</sub> and CH<sub>4</sub> was examined in samples collected at 4 h intervals from 00:00 to 24:00 h, on 15 August 2014. Bottom-water samples were collected using a 2.5 l polymethyl methacrylate water sampler (Yuanda Nikkor), approximately 10–20 cm above the sediment layers, and transported to the laboratory for incubation. Sediment samples were collected with a core sampler (50 mm in diameter × 35 cm in length; Wang & Xu 2004), and transported to the laboratory for incubation and analyses of environmental parameters, including water temperature, dissolved oxygen (DO), pH, dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), and particulate organic carbon (POC) in the overlying water, and oxidation–reduction potential (Eh), OM, and total carbon (TC) in the surface sediment.

### Laboratory incubation and analyses of LLGHGs

The CO<sub>2</sub> and CH<sub>4</sub> fluxes across the sediment–water interface were determined by *ex situ* incubation. All sediment samples were brought to the laboratory within 1 h after collection and left for 2 h in the incubation chambers to recover equilibrium. After equilibration, the overlying

water in each chamber was carefully replaced with bottom water using a rubber pipe, maintaining the water flow low enough to avoid any disturbance of the sediment surface. When chambers were filled with bottom water, cores were sealed with rubber stoppers. Two magnets, one placed at the upper lid of each chamber plus a central magnet (Fig. 1), allowed stirring of samples at 50 rpm during the water-bath incubation, performed in the darkness, for 4 h, at the *in situ* temperature (May, 21.33°C; June, 28.37°C; July, 30.60°C; August, 32.53°C; and September, 24.31°C). Two samples containing only bottom water were incubated in the same conditions and used as controls.

Parameter	GSBT	GSBP	GSBC
Stocking (kg m <sup>-3</sup> )	0.57	0.58	0.57
Stocking density (ind. m <sup>-3</sup> )	G:1.74; S:0.14; B:0.04; T:0.14	G:1.76; S:0.15; B:0.04; P:35.51	G:1.78; S:0.14; B:0.04; C:0.46
Yield (all species; kg m <sup>-3</sup> )	2.72	3.15	3.11
Feed input (kg m <sup>-3</sup> )	3.97	4.14	4.06
Transparency (m)	0.22 ± 0.09	0.27 ± 0.18	0.20 ± 0.07
Phosphate (mg l <sup>-1</sup> )	0.65 ± 0.31	0.81 ± 0.27	0.93 ± 0.18
Ammonia (mg l <sup>-1</sup> )	1.21 ± 0.98	1.46 ± 1.14	1.72 ± 1.25

Temperature and DO concentration in the overlying water were determined using a digital DO meter (YSI 550), and pH was measured using an acidometer (PHS-3C; Shanghai REX Instruments). The redox potential (Eh) of surface sediment was measured

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Table 2. Starting conditions in the sediment of polyculture systems GSBT, GSBP, and GSBC (see Table 1 for abbreviations). Eh: redox potential. Means ± SD

	GSBT	GSBP	GSBC
Eh (mV)	-136.17 ± 9.93	-132.67 ± 7.97	-139.83 ± 9.02
Organic matter (%)	3.82 ± 0.27	4.15 ± 0.35	3.95 ± 0.32
Total carbon (mg g <sup>-1</sup> )	24.56 ± 0.53	24.12 ± 0.28	23.62 ± 0.53
Total nitrogen (mg g <sup>-1</sup> )	2.48 ± 0.17	2.16 ± 0.18	2.42 ± 0.19

with an oxidation–reduction potentiometer (AZ8551; AZ Instruments). Sediment samples were dried at 60°C until all water was evaporated, and then sieved through a 1 mm mesh. OM contents were measured as loss on ignition (LOI) after combustion at 450°C in a muffle furnace for at least 5 h. DOC and DIC in the overlying water were analyzed in a total organic carbon (TOC) analyzer (multi N/C<sup>®</sup> 2100; Analytik Jena), after passing through pre-combusted (450°C, 2 h) Whatman GF/F-filters. Filtered POC in the overlying water and sediment TC were determined with a CHONS elemental analyzer (Vario ELIII; Elementar Analysensysteme).

Water samples (100 ml) were taken from the sediment surface-water column within each incubation chamber, and transferred to headspace vials to determine dissolved CO<sub>2</sub> and CH<sub>4</sub> concentrations using gas chromatography based on gas-stripping methods (Zhang et al. 2010). Within each vial, 50 ml of the sample were replaced with nitrogen, shaken vigorously for 5 min, and then allowed to equilibrate for at least 2 h. Finally, the headspace gas was analyzed with a gas chromatograph (GC-2010 Plus; Shimadzu) equipped with a TDX (2 m × 2 mm, 60–80 mesh) column. The CO<sub>2</sub> gas samples were placed into the MGS-4 gas sampler (a manual gas sampler for the GC-2010 Plus), and converted into CH<sub>4</sub> in the MTN-1 methanizer using a nickel catalyst at 375°C, after being separated from the TDX column at 40°C. The concentrations of CO<sub>2</sub> and CH<sub>4</sub> were then deter-

mined with a flame ionization detector at 220 and 90°C, respectively. Carrier gas (nitrogen) flow rate was 22 ml min<sup>-1</sup>, and flame gases (hydrogen and compressed air) flow rates were set at 20 and 30 ml min<sup>-1</sup>, respectively. Standard gases were measured every 4 runs to determine sample concentrations and check for errors. Precisions of repeated analysis of water samples were about 5 % for CO<sub>2</sub> and 3 % for CH<sub>4</sub>.

Dissolved CO<sub>2</sub> and CH<sub>4</sub> concentrations in water samples were calculated applying Henry's law and taking solubility dependence upon temperature into account (Lide & Frederikse 1997). Measured CO<sub>2</sub> and CH<sub>4</sub> concentrations in the overlying water were compared to their theoretical concentrations in equilibrium with the atmosphere. The atmospheric concentrations used in calculations were the seasonal average air concentrations of CO<sub>2</sub> and CH<sub>4</sub>, which were sampled with polypropylene syringes 1 m above the pond surface, during chamber measurements.

The CO<sub>2</sub> and CH<sub>4</sub> fluxes across the sediment–water interface were calculated using Eq. (1) (Zheng et al. 2009, Zhong et al. 2015a,b):

$$F = \frac{\Delta C \times V}{A \times t} \quad (1)$$

where  $F$  is the CO<sub>2</sub> or CH<sub>4</sub> flux (mmol m<sup>-2</sup> d<sup>-1</sup>),  $\Delta C$  is the variation in CO<sub>2</sub> or CH<sub>4</sub> concentration measured before and after incubation, after being corrected according to atmospheric CO<sub>2</sub> or CH<sub>4</sub> concentration in the control incubators (mmol l<sup>-1</sup>),  $V$  is the volume

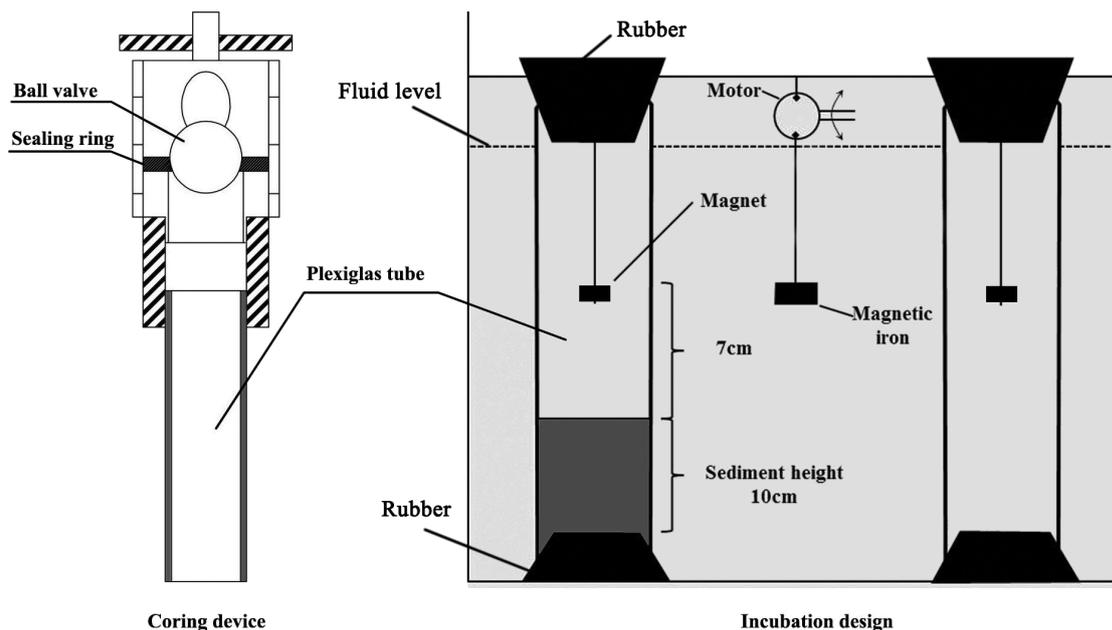


Fig. 1. Sketch of the coring device (50 mm diameter × 35 cm long) used to extract sediment samples from 3 different experimental polyculture systems (see Table 1). Also shown is the general design for sediment incubation in the laboratory. The incubation and analyses are described in detail in 'Laboratory incubation and analyses of LLGHGs'

of the incubator ( $m^3$ ),  $A$  is the bottom area of the incubator ( $m^2$ ), and  $t$  is the incubation duration (d). Negative fluxes of  $CO_2$  or  $CH_4$  indicated sediment  $CO_2$  or  $CH_4$  absorption from the water, whereas positive fluxes indicated that  $CO_2$  or  $CH_4$  were released into the water–sediment interface.

### Statistical analysis

Analyses were performed in SPSS for Windows 21.0. Means were compared using analysis of variance (ANOVA), followed by Duncan's multiple range tests for post hoc comparisons. The fluxes of  $CO_2$  and  $CH_4$  were correlated to environmental factors using Pearson's correlation method.

## RESULTS

### Overlying water and surface sediment characteristics

The overlying water characteristics determined for the 3 polyculture systems are shown in Fig. 2a–f. Overlying water temperature varied between 21.30 and 32.63°C, peaking in August (Fig. 2a). Minimum DO concentrations were registered in August and maximum concentrations in May, and average values differed significantly between polyculture systems from June to September (Fig. 2b). The presence of *Litopenaeus vannamei* and *Cyprinus carpio* tended to decrease DO concentration in the overlying water. A similar trend was obtained for pH, with GSBC presenting the lowest values and GSBT the highest

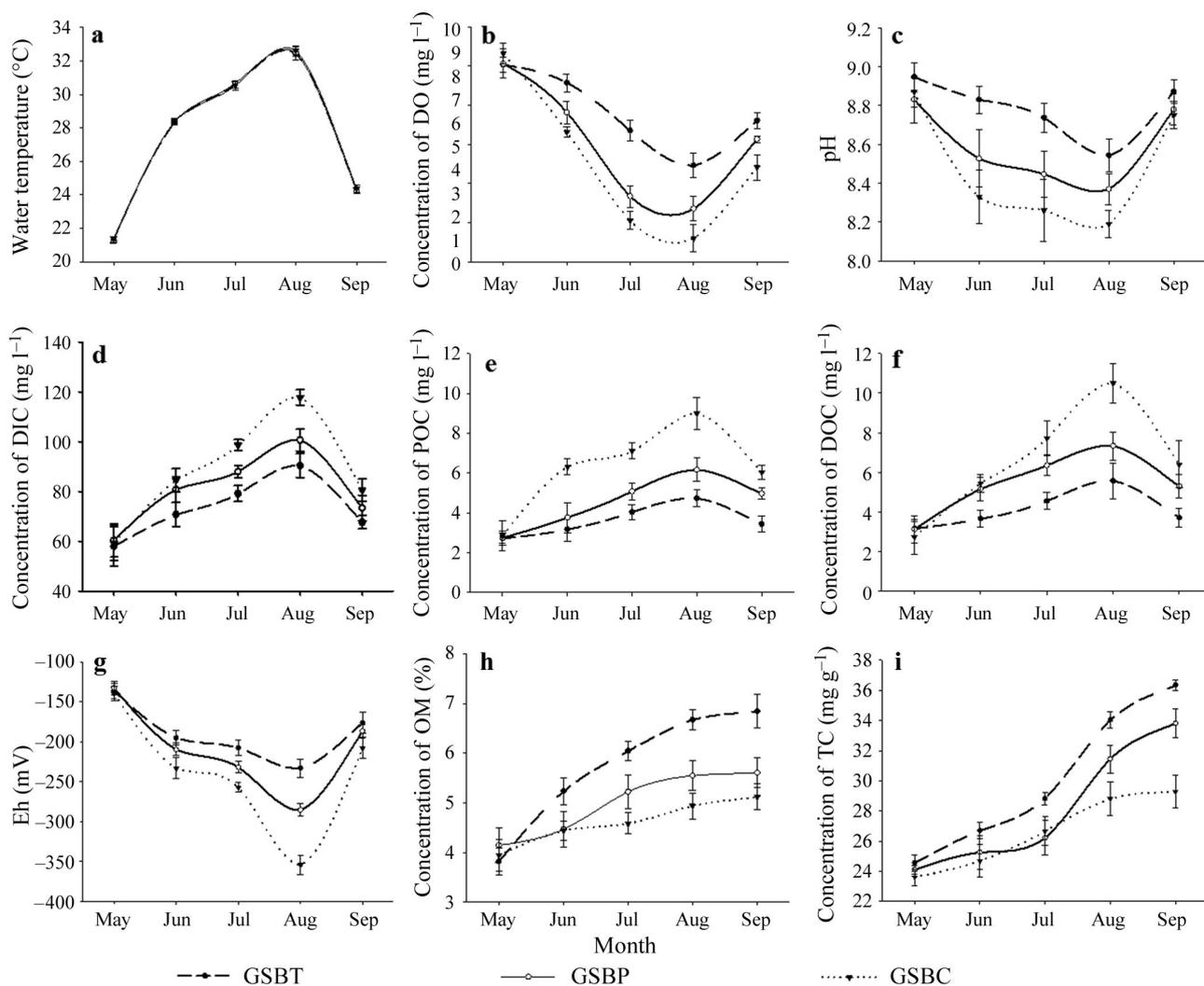


Fig. 2. Monthly variations in (a) temperature, (b) dissolved oxygen (DO), (c) pH, (d) dissolved inorganic carbon (DIC), (e) particulate organic carbon (POC), and (f) dissolved organic carbon (DOC) in the overlying water and (g) redox potential (Eh), (h) organic matter (OM), and (i) total carbon (TC) in the surface sediment of GSBT, GSBP, and GSBC experimental polyculture ponds (see Table 1 for polyculture system abbreviations)

(Fig. 2c). The DIC concentration in the overlying water increased gradually from May to August (Fig. 2d). The highest values were observed in GSBC ponds in July and August, and were remarkably higher than that in GSBT and GSBP ponds, within the same period. Similar trends were observed for POC and DOC, whose highest values were observed in GSBC ponds and lowest in GSBT ponds (Fig. 2e,f). Therefore, the presence of *L. vannamei* and *C. carpio* seemed to increase DIC, DOC, and POC concentrations in the overlying water.

The surface sediment characteristics for the 3 polyculture systems are shown in Fig. 2g–i. The minimum Eh occurred in August and the maximum in May, and significant differences were detected among polyculture systems in July and August (Fig. 2g). The presence of *L. vannamei* and *C. carpio* tended to decrease Eh in the surface sediment. Both OM and TC accumulated in the sediments of the 3 polyculture systems throughout the experiment (Fig. 2h,i), with GSBT ponds presenting the highest OM and TC concentrations from June to September.

#### Fluxes of CO<sub>2</sub> and CH<sub>4</sub> at the sediment–water interface

Average CO<sub>2</sub> fluxes (in mmol m<sup>-2</sup> d<sup>-1</sup>) at the sediment–water interface were 24.19 (range: 10.86–37.08), 35.15 (12.25–51.95), and 43.58 (12.67–67.71) in GSBT, GSBP, and GSBC ponds, respectively (Fig. 3a). The CO<sub>2</sub> fluxes in the 3 polyculture systems increased gradually from May to August, peaking in August. No significant differences were observed among culture

systems in May ( $p > 0.05$ ), but CO<sub>2</sub> fluxes were significantly higher in GSBC than in the other 2 systems in July and August ( $p < 0.05$ ). The CO<sub>2</sub> fluxes were significantly higher in GSBP than in GSBT ( $p < 0.05$ ), except for May and September (Fig. 3a).

Average CH<sub>4</sub> fluxes (in mmol m<sup>-2</sup> d<sup>-1</sup>) at the sediment–water interface were 0.58 (range: 0.27–1.37) in GSBT, 1.16 (0.25–2.76) in GSBP, and 2.26 (0.37–5.29) in GSBC (Fig. 3b). In all culture systems, CH<sub>4</sub> fluxes increased from May to August and decreased in September (Fig. 3b). Throughout the experiment, CH<sub>4</sub> fluxes were consistently higher in GSBC than in GSBT and GSBP, except in May ( $p > 0.05$ ), and significantly higher in GSBP than in GSBT from July to September ( $p < 0.05$ ; Fig. 3b).

#### Diel variation in CO<sub>2</sub> and CH<sub>4</sub> concentrations

Within 24 h, the CO<sub>2</sub> concentration in the overlying water varied from 37.46 to 77.19 mmol l<sup>-1</sup> in GSBT, 60.31 to 119.31 mmol l<sup>-1</sup> in GSBP, and 71.34 to 112.59 mmol l<sup>-1</sup> in GSBC (Fig. 4a). The CO<sub>2</sub> concentrations increased gradually from 16:00 to 00:00 h, peaked at 04:00 h, and gradually decreased thereafter. From 16:00 to 12:00 h, CO<sub>2</sub> concentrations were significantly higher in GSBP and GSBC than in GSBT ( $p < 0.05$ ), except at 20:00 h. At 8:00 and 12:00 h, CO<sub>2</sub> concentrations were significantly higher in GSBC than in GSBP ( $p < 0.05$ ; Fig. 4a).

Diel variations of the CH<sub>4</sub> concentration in the overlying water of the 3 culture systems are shown in Fig. 4b. The CH<sub>4</sub> concentrations (in mmol m<sup>-2</sup> d<sup>-1</sup>) ranged from 0.77 to 2.11 in GSBT, 1.44 to 3.53 in

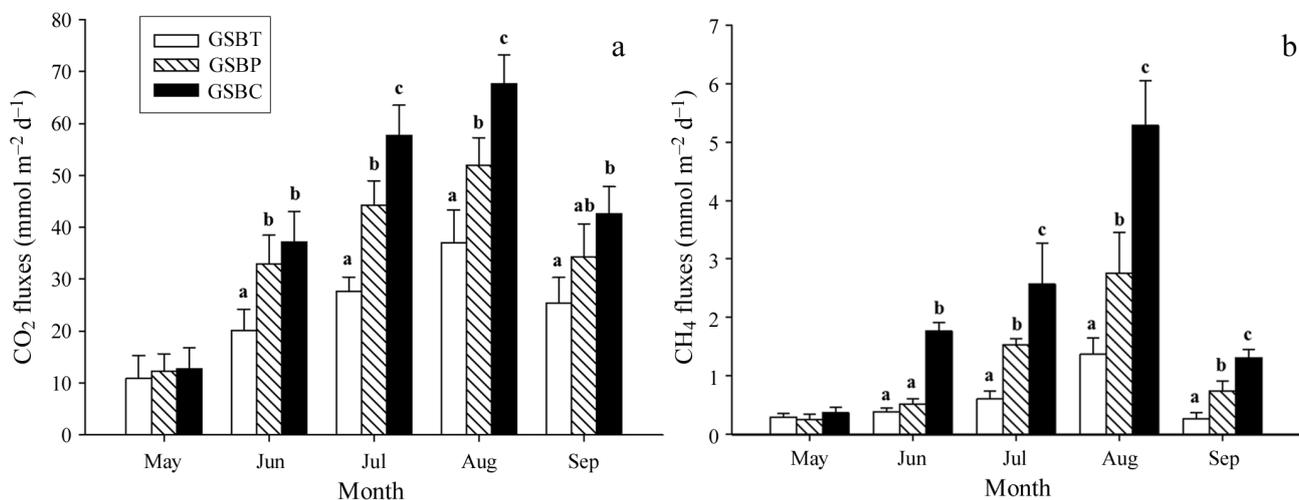


Fig. 3. (a) Carbon dioxide and (b) methane fluxes across the sediment–water interface in the 3 experimental polyculture systems (see Table 1) from May to September 2014. Bars denote standard deviation ( $n = 3$ ). Different letters indicate significant differences at  $p < 0.05$ , according to ANOVA and Duncan's multiple range tests

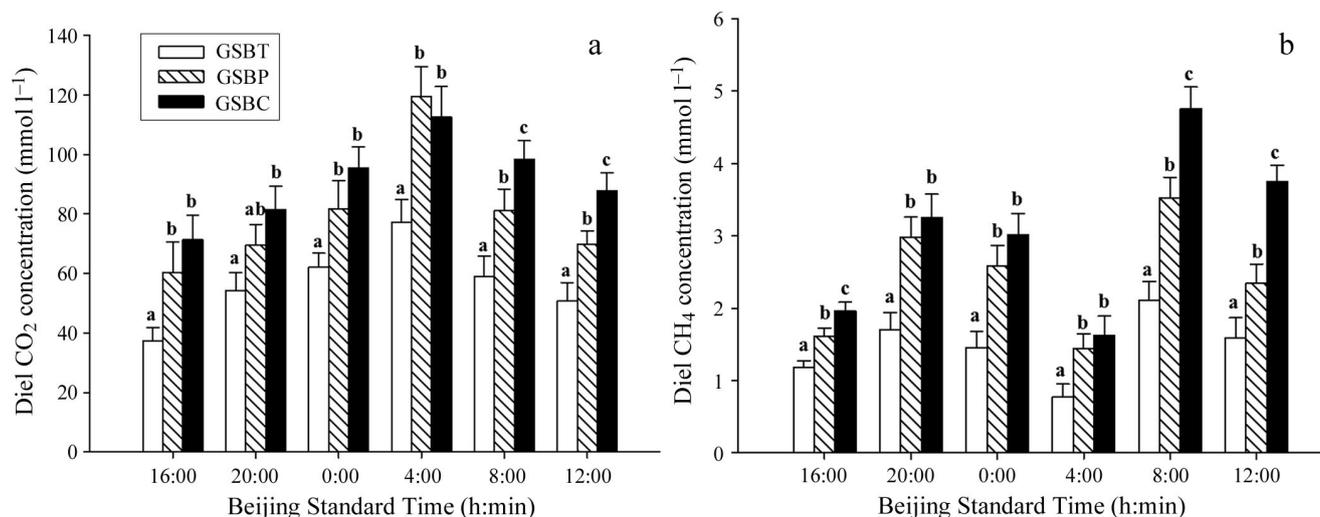


Fig. 4. Diel variation in (a) carbon dioxide and (b) methane concentrations in the overlying water of the 3 experimental polyculture systems (see Table 1) in August 2014. Bars denote standard deviation ( $n = 3$ ). Different letters indicate significant differences at  $p < 0.05$  by ANOVA and Duncan's multiple range tests

GSBP, and 1.62 to 4.76 in GSBC. The minimum  $\text{CH}_4$  concentrations occurred at 04:00 h and the maximum at 08:00 h. Over a daily cycle,  $\text{CH}_4$  concentrations were significantly lower in GSBT than in GSBP and GSBC ( $p < 0.05$ ). At 08:00, 12:00, and 16:00 h,  $\text{CH}_4$  concentrations were significantly higher in GSBC than in GSBP ( $p < 0.05$ ), with no significant differences at any other time.

#### Correlation between $\text{CO}_2$ and $\text{CH}_4$ fluxes and environmental factors

According to Pearson's correlations (Table 3),  $\text{CO}_2$  and  $\text{CH}_4$  fluxes were negatively correlated with DO concentrations and pH in the overlying water, and with surface sediment Eh; positive correlations were found for temperature, DIC, POC, and DOC in the overlying water, and OM and TC in the surface sediment.

## DISCUSSION

### Fluxes of $\text{CO}_2$ and $\text{CH}_4$ in aquaculture ponds and other aquatic ecosystems

Carbon dioxide and  $\text{CH}_4$  fluxes across the sediment–water interface have been measured in different ecosystems, including oceans, rivers, reservoirs, and lakes (Moosavi et al. 1996, Liikanen & Martikainen 2003, Adams 2005, Huttunen et al. 2006), but, to our knowledge, not in freshwater aquaculture systems. The range and average values of  $\text{CO}_2$  fluxes determined in the 3 *Ctenopharyngodon idella* polyculture systems evaluated in the present study (Table 4), were higher than those reported for lakes and reservoirs, but generally lower than those reported for drainage ditches and intertidal mudflats (Kikuchi 1986, Adams 2005, Schrier-Uijl et al. 2011). Adams (2005) reported that  $\text{CO}_2$  fluxes

Table 3. Pearson correlation coefficients between  $\text{CO}_2$  and  $\text{CH}_4$  fluxes and factors in 3 polyculture systems (see Table 1 for abbreviations) during the farming season. T: temperature, DO: dissolved oxygen DIC: dissolved inorganic carbon, POC: particulate organic carbon, DOC: dissolved organic carbon, Eh: redox potential, OM: organic matter, TC: total carbon. \* $p < 0.05$ ; \*\* $p < 0.01$

System		T	DO	pH	DIC	POC	DOC	Eh	OM	TC
GSBT	$\text{CO}_2$	0.785**	-0.845**	-0.654**	0.721**	0.718**	0.742**	-0.830**	0.794**	0.678**
	$\text{CH}_4$	0.761**	-0.743**	-0.805**	0.769**	0.776**	0.758**	-0.747**	0.435	0.368
GSBP	$\text{CO}_2$	0.892**	-0.906**	-0.710**	0.918**	0.868**	0.891**	-0.926**	0.645**	0.468
	$\text{CH}_4$	0.799**	-0.826**	-0.674**	0.864**	0.945**	0.752**	-0.890**	0.592*	0.434
GSBC	$\text{CO}_2$	0.892**	-0.941**	-0.795**	0.930**	0.939**	0.898**	-0.890**	0.614*	0.701**
	$\text{CH}_4$	0.877**	-0.794**	-0.743**	0.911**	0.907**	0.846**	-0.934**	0.429	0.500

Table 4. Comparisons of CO<sub>2</sub> fluxes (mmol m<sup>-2</sup> d<sup>-1</sup>) across the water–sediment interface in different aquatic ecosystems (n = no. of ecosystems sampled)

Site	n	Average depth (m)	Mean	Range	Reference
Aquaculture ponds, China	9	1.8	34.31	10.80–67.68	Present study
Oligotrophic lakes	4	19–98	0.34	–2.39–2.46	Adams (2005)
Mesotrophic lakes	5	5–21.6	2.60	1.47–3.93	Adams (2005)
Eutrophic lakes	5	4–46	3.80	0.74–8.57	Adams (2005)
Eutrophic reservoirs	2	25.6	4.26	–0.06–17.70	Adams (2005)
Lobo Broa Reservoir, Brazil	1	3	15.88	8.80–26.05	Adams (2005)
Drainage ditches, Netherlands	7	0.25–0.90	70.41	37.96–108.53	Schrier-Uijl et al. (2011)
Nanakita River intertidal mudflats, Japan	1	–	166.00	64.00–291.00	Kikuchi (1986)

across the sediment–water interface progressively increased with increasing trophic levels in natural aquatic systems. Furthermore, a negative correlation between nutrient levels and the water depth and size of aquatic ecosystems has been reported (Odum & Barrett 2005). Hence, regarding lakes or reservoirs, aquaculture ponds, drainage ditches, and intertidal mudflats as ecosystems with gradually increasing nutrient levels, the values found for CO<sub>2</sub> fluxes across the sediment–water interface in the present study support the premise that in freshwater systems, these fluxes increase with increasing nutrient levels.

The range and average values of CH<sub>4</sub> fluxes in the 3 *C. idella* polyculture systems examined here (Table 5) were higher than those reported for oligotrophic lakes and reservoirs, but generally lower than those observed in mesotrophic and eutrophic lakes or reservoirs and drainage ditches (Adams 2005, Schrier-Uijl et al. 2011). Although CH<sub>4</sub> fluxes increased with increasing trophic levels in natural aquatic systems (Adams 2005), the values obtained in this study were lower than those reported for eutrophic lakes and reservoirs. This might be due to the DO (4.60–9.32 mg l<sup>-1</sup>) produced by artificial aer-

ation, which led to the rapid oxidation of dissolved CH<sub>4</sub> in freshwater aquaculture ponds during the experiment. Accordingly, Conrad & Rothfuss (1991) found that approximately 80% of the CH<sub>4</sub> diffusing through the oxidized sediment–water interface was consumed by methanotrophs, which were limited by the O<sub>2</sub> availability in methanogenic environments.

#### Correlation between CO<sub>2</sub> and CH<sub>4</sub> fluxes and environmental factors

Microbial activity is an important source of CO<sub>2</sub> and CH<sub>4</sub> in aquatic systems, and CO<sub>2</sub> and CH<sub>4</sub> fluxes can be affected by environmental factors such as water temperature, DO, pH, and Eh (Cicerone & Shetter 1981, Crozier et al. 1995, Liikanen et al. 2002, Ding & Cai 2003, Tan 2014). Correlation analysis showed that the CO<sub>2</sub> and CH<sub>4</sub> fluxes in all polyculture systems were positively correlated with water temperature (Table 3), which was consistent with previous indications that CO<sub>2</sub> and CH<sub>4</sub> concentrations in water increase with increasing water temperature (Moore & Dalva 1993, Hamilton et al. 1995, den Heyer & Kalff 1998). These positive correlations might be explained

Table 5. Comparisons of CH<sub>4</sub> fluxes (mmol m<sup>-2</sup> d<sup>-1</sup>) across the water–sediment interface in different aquatic ecosystems (n = no. of ecosystems sampled)

Site	n	Average depth (m)	Mean	Range	Reference
Aquaculture ponds, China	9	1.8	1.33	0.25–5.29	Present study
Oligotrophic lakes	4	19–98	0.19	0.02–0.57	Adams (2005)
Oligotrophic reservoir	1	40	0.39	0.38–0.39	Adams (2005)
Mesotrophic lakes	5	5–21.6	2.07	0.0–4.39	Adams (2005)
Eutrophic lakes	5	4–46	3.90	0.01–12.00	Adams (2005)
Eutrophic reservoirs	2	25.6	5.24	0.20–19.27	Adams (2005)
Lobo Broa Reservoir, Brazil	1	3	8.67	5.34–15.00	Adams (2005)
Drainage ditches, Netherlands	7	0.25–0.90	50.55	1.8–58.95	Schrier-Uijl et al. (2011)

by the increase in microbial activity and carbon mineralization caused by temperature increases, resulting in an oversaturation of CO<sub>2</sub> and CH<sub>4</sub> and their subsequent release from the sediment to the overlying water.

The high negative correlation observed between DO and Eh levels and CO<sub>2</sub> and CH<sub>4</sub> fluxes (Table 3) probably resulted from CO<sub>2</sub> production during aerobic respiration and concomitant O<sub>2</sub> consumption (Hamilton et al. 1995, Ballester & dos Santos 2001). In addition, methanogens are obligate anaerobes, and DO content not only inhibits CH<sub>4</sub> production, but also oxidizes the CH<sub>4</sub> released from the sediment to the overlying water (Conrad 1996, Dernier Van der Gon & Neue 1996, Bosse & Frenzel 1998, Frenzel & Karofeld 2000). Decreasing Eh levels might also have led to a boost in CH<sub>4</sub> release in freshwater aquaculture ponds, as it was reported that a decrease in Eh from -200 to -300 mV induced a 10-fold increase in CH<sub>4</sub> production and a 17-fold increase in its release in a rice field (Kludze et al. 1993).

The high negative correlation coefficients between pH and CO<sub>2</sub> and CH<sub>4</sub> fluxes (Table 3) supported the importance of this environmental variable pointed out in previous studies (Neal et al. 1998, Waldron et al. 2007, Crawford et al. 2013). Given that CO<sub>2</sub> is easily dissolved in water, forming carbonic acid, a low pH indicates a high CO<sub>2</sub> content in the water, and high CO<sub>2</sub> diffusion gradients and efflux between the sediment and the overlying water. Similarly, Wallin et al. (2013) reported that CO<sub>2</sub> partial pressure was higher at low pH, but greatly decreased at high pH. Thus, the correlations observed in the present study were probably due to all of the above-mentioned effects. In addition, the optimum pH of CH<sub>4</sub> production was near neutrality, and a slight decrease in overlying water pH resulted in the enhancement of CH<sub>4</sub> production and release (Wang et al. 1993).

In addition to water temperature, DO, pH, and Eh, the OM and TC contents were important factors determining the CO<sub>2</sub> and CH<sub>4</sub> fluxes, as shown by the high positive correlation coefficients obtained between the fluxes of these gases and OM and TC (Table 3). Because carbon provides the substrate necessary for microbial CO<sub>2</sub> and CH<sub>4</sub> formation (Adams 2005, Beaulieu et al. 2009, Atkins et al. 2013), high positive correlations were observed between DIC, POC, and DOC and CO<sub>2</sub> and CH<sub>4</sub> fluxes (Table 3). Several studies revealed that the continuous enrichment of OM and TC increases DIC, POC, and DOC in aquatic systems (Li 2004, Yokoyama et al. 2006, Valdemarsen et al. 2009). Therefore, carbon concentration in the overlying water might reflect OM degradation rate across the water-sediment interface.

### Differences among the 3 polyculture systems

In previous carbon flux and carbon form distribution experiments performed on grass carp polyculture systems (Xiong et al. 2015, 2016), we found that *Litopenaeus vannamei* and *Cyprinus carpio* tended to increase the transport and conversion of carbon from the sediment to the overlying water in a different pattern. In those studies, the carbon released from the sediment while bottom feeders were burrowing was found to be particularly important for the common carp. The increasing CO<sub>2</sub> and CH<sub>4</sub> fluxes in the sediment-water interface in GSBP and GSBC systems found in the present study corroborated the previous results, as they affected some environmental factors that are most important for bottom feeders. In addition, not finding significant increases in CO<sub>2</sub> and CH<sub>4</sub> fluxes in the GSBT system suggested that benthic respiration was important to release CO<sub>2</sub> and CH<sub>4</sub> (King et al. 1990, Jansson et al. 2003). The higher DO concentration and Eh found in this system compared to those in GSBP and GSBC systems might have led to a faster oxidation of dissolved CH<sub>4</sub>, which is supported by the significant decrease in DO and pH in the overlying water and Eh in the surface sediment of GSBP and GSBC systems (Fig. 2b,c,g). Thus, the metabolism of *L. vannamei* and *C. carpio* might be important to decrease DO, pH, and Eh levels.

In fact, the release of CO<sub>2</sub> and CH<sub>4</sub> trapped in the sediment by *L. vannamei* and *C. carpio* was an important mechanism identified in the present study. Strong disturbances in the upper sediment layers by *L. vannamei* and *C. carpio* were observed during the experiment, and resulted in the significant increase of POC, DOC, and DIC in the overlying water of GSBP and GSBC systems (Fig. 2d-f). The higher water turbidity due to sediment disturbance might have reduced phytoplankton photosynthesis, leading to the low DO concentrations, pH, and Eh observed in GSBP and GSBC systems (Fig. 2b,c,g) (Frei et al. 2007). The contents of OM and TC in the sediment (Fig. 2h,i) reflected CO<sub>2</sub> and CH<sub>4</sub> release across the water-sediment interface, with GSBT having the highest accumulation of OM and TC and the lowest CO<sub>2</sub> and CH<sub>4</sub> release. The enhanced OM degradation rate due to *L. vannamei* and *C. carpio* bioturbation was accompanied by a decrease in oxygen levels and increase in CO<sub>2</sub> and CH<sub>4</sub> release.

In the present study, CO<sub>2</sub> fluxes were significantly higher in GSBC than in GSBP during July and August, and CH<sub>4</sub> fluxes were significantly higher in GSBC than in GSBP in all months, except in May

(Fig. 3). These relationships might be explained by differences in *L. vannamei* and *C. carpio* bioturbation. Some studies suggested that *L. vannamei* in a *C. carpio* polyculture pond might affect the transport and conversion of carbon in surface sediment, as *L. vannamei* sediment bioturbation depth was about 0 to 2 cm (Guo et al. 2012, Zhong et al. 2015a) while that of *C. carpio* was about 1 to 5 cm (Zhong et al. 2015b). Therefore, these species had different impacts on the CO<sub>2</sub> and CH<sub>4</sub> fluxes, as they affected overlying water and sediment characteristics differently.

It is also important to note the difference between diel variations in CO<sub>2</sub> and CH<sub>4</sub> concentrations in the overlying water of the 3 polyculture systems during August: CO<sub>2</sub> and CH<sub>4</sub> concentrations were significantly higher in GSBC than in GSBP at 08:00 and 12:00 h, but there were no significant differences from 20:00 to 04:00 h. This might be due to *L. vannamei* being more active during the night, whereas *C. carpio* were more active during the day, therefore disturbing the sediment at different times. Thus, *C. carpio* bioturbation seems to promote OM decomposition during daytime. Based on the results of the present study, we suggest that the diel variations in CO<sub>2</sub> and CH<sub>4</sub> concentrations are mainly affected by the behavior of benthic stocked organisms, which is also consistent with the CO<sub>2</sub> and CH<sub>4</sub> fluxes obtained in the 3 polyculture models examined here.

## CONCLUSIONS

In summary, the results obtained here indicated that CO<sub>2</sub> fluxes ranged from 10.86 to 67.71 mmol m<sup>-2</sup> d<sup>-1</sup>, while CH<sub>4</sub> fluxes varied from 0.25 to 5.29 mmol m<sup>-2</sup> d<sup>-1</sup> during the culture period. These fluxes were negatively correlated with DO concentrations and pH in the overlying water and with surface sediment Eh, and positively correlated with temperature, DIC, POC, and DOC in the overlying water, and OM and TC in the surface sediment. In addition, stocking *Litopenaeus vannamei* and *Cyprinus carpio* in polyculture models could promote the release of CO<sub>2</sub> and CH<sub>4</sub> from the sediment to the overlying water, and the different bioturbation depths of these species could affect biogeochemical processes.

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