Methane dynamics in an estuarine brackish *Cyperus malaccensis* marsh: Production and porewater concentration in soils, and net emissions to the atmosphere over five years

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A B S T R A C T

Wetlands can potentially affect global climate change through their role in modulating the atmospheric concentrations of methane (CH_4). Their overall CH_4 emissions, however, remain the greatest uncertainty in the global CH_4 budget. One reason for this is the paucity of long-term field measurements to characterize the variability of CH_4 emissions from different types of wetlands. In this study, we quantified CH_4 emissions from a brackish, oligohaline *Cyperus malaccensis* marsh ecosystem in the Min River Estuary in southeast China over five years. Our results showed substantial temporal variability of CH_4 emissions from this brackish marsh, with hourly fluxes ranging from 0.7±0.6 to 5.1±3.7 mg m^{-2} h^{-1} (mean ± 1 SD) during the study period. The inter-annual variability of CH_4 emissions was significantly correlated with changes in soil temperature, precipitation and salinity, which highlighted the importance of long-term observations in understanding wetland CH_4 dynamics. Distinct seasonal patterns in soil CH_4 production rates and porewater CH_4 concentrations also were observed, and were both positively correlated with CH_4 emissions. The seasonal variations of CH_4 emissions and production were highly correlated with salinity and porewater sulfate levels. The mean annual CH_4 efflux from our site over the five-year period was 23.8±18.1 g CH_4 m^{-2} yr^{-1}, indicating that subtropical brackish tidal marsh ecosystems could release a large amount of CH_4 into the atmosphere. Our findings further highlight the need to obtain high-frequency and continuous field measurements over the long term at multiple spatial scales to improve our current estimates of wetland CH_4 emissions.

Keywords: Methane; Net emissions; Soil production; Porewater; Temporal variation; Estuarine marsh

1. Introduction

The increasing worldwide concern over global climate change and its effects on environmental and human well-beings calls for a better understanding of the magnitude of global greenhouse gas emissions (Tong et al., 2010). Methane (CH_4) is a potent greenhouse gas with a global warming potential 34 times higher than that of CO_2 over a 100-year time scale, and contributes to approximately 20% of the global radiative forcing (IPCC, 2013). Global atmospheric CH_4 levels have increased by threefold since 1750, reaching 1845±2 ppb in 2015 (World Meteorological Organization, 2016). Quantifying the potential source strength of various ecosystems has become one of the top priorities for improving the future predictions of CH_4 emissions.

Wetlands are estimated to contribute 20–39% of the global CH_4 emissions (Laanbroek, 2010), with natural wetlands being the single largest source of CH_4. Over the past few decades, considerable efforts were made to quantify CH_4 emissions from different natural wetlands around the world (e.g. Bubier et al., 1994; Kutzbach et al., 2004; Hendriks et al., 2010; Tong et al., 2012). However, the majority of these field campaigns were carried out over a relatively short period of not more than two years, which provided little knowledge of the inter-annual variability of CH_4 emissions from most types of wetlands other than a few exceptions in northern wetlands, e.g. Song et al. (2009), Jackowicz-Korczyński et al. (2010), and Moore et al. (2011). Long-term observations over multiple seasons and years are critical for determining accurate ecosystem CH_4 budgets (Song et al., 2009). In addition, the availability of a long-term data set will improve ecosystem modelling by providing inputs for model calibration and validation, as well as insights on the key factors regulating wetland CH_4 emissions into the atmosphere (Tian et al., 2008; Song

et al., 2009).

Coastal wetlands, located at the interface between the terrestrial and marine environments, are biogeochemically important ecosystems that span widely from the arctic to the tropical zones (Chmura et al., 2003; Wang et al., 2016). Previous studies have shown that the sediments in coastal wetlands are generally small atmospheric sources (Bartlett & Harriss, 1993; Poffenbarger et al., 2011; Livesley & Andrusiak, 2012; Koebsch et al., 2013), or even weak sinks of CH_4 (Sun et al., 2013). The low CH_4 source strength of coastal wetlands is mainly because of the relatively high sulfate concentrations in marine waters, which favour the activities of sulfate-reducing bacteria while at the same time hamper the metabolism of methanogens through intense competition for substrates (Poffenbarger et al., 2011; Callaway et al., 2012; Vizza et al., 2017). However, some short-term field studies provide evidence that large CH_4 emissions from wetlands can occur even when sulfate reduction is a dominant process (Lee et al., 2008; Marín-Muñiz et al., 2015; Holm Jr. et al., 2016). The high uncertainty associated with the magnitude and control of CH_4 emissions from coastal wetlands could partly be related to the inherently dynamic environment which introduces a large temporal variability of CH_4 fluxes that is not adequately accounted for by some infrequent field measurements.

In this study, monthly CH_4 flux measurements were made in a subtropical tidal *Cyperus malaccensis* (shichito matgrass) marsh in the Min River Estuary in southeast China over five years between 2007-2009, and 2013-2014. We hypothesized that there would be significant seasonal and inter-annual variability in CH_4 emissions, which implies that flux estimates would be sensitive to the sampling frequency and study duration. We also investigated the temporal correlations between several environmental variables with soil CH_4 production rate, porewater

90 CH_4 concentration, and net CH_4 emissions.

91 2. Materials and methods

92 *2.1. Site description*

93 This study was carried out in the Shanyutan wetland (26°00′36″–26°03′42″ N,
94 119°34′12″–119°40′40″ E), the largest tidal wetland area (ca. 3120 ha) in the Min River Estuary,
95 southeast China (Fig. 1). The Shanyutan wetland is influenced by a subtropical monsoonal
96 climate, with a mean annual temperature of 19.6 °C and an annual precipitation of 1350 mm
97 (Tong et al., 2010). The dominant vegetation species in the Shanyutan wetland included the
98 native *Cyperus malaccensis* and *Phragmites australis*, as well as the invasive *Spartina*
99 *alterniflora* (smooth cordgrass). The average height of *C. malaccensis* at the site was about 1.4 m.
100 The study site was characterized by semi-diurnal tides, such that the soil surface was submerged
101 for approximately 7 h over a 24 h cycle, and at other times, the soil surface was exposed to air
102 (Tong et al., 2010). The average salinity of the tidal water was 4.2±2.5‰ (Tong et al., 2010).

103 *2.2. Gas sampling and CH_4 flux estimation*

104 Net CH_4 emissions were measured in the intertidal zone in the mid-western part of the
105 Shanyutan wetland (26°01′46″ N, 119°37′31″ E), which was dominated by *C. malaccensis*, a
106 widespread plant species at the site. Triplicate 1 m x 1 m plots, with a distance of < 5 m between
107 plots, were established for regular measurement of CH_4 emissions in the *C. malaccensis* stand.
108 CH_4 flux measurements were carried out monthly from early January to early December in
109 2007–2009 and 2013–2014. A wooden boardwalk was built to facilitate access to the study plots
110 and minimize potential plot disturbance caused by field measurements. The wooden boardwalk
111 and the study plots were damaged during a major typhoon event in 2010, thus we built a new

boardwalk and established new plots adjacent to the damaged ones (< 15 m apart) in 2012. During 2013–2014, we continued with gas flux measurements at the new plots.

CH_4 flux measurements were made using static closed chambers and gas chromatography techniques (Hirota et al., 2004; Song et al., 2009; Moore et al., 2011; Marín-Muñiz et al., 2015) with gas samples collected during the neap tides in the morning. The static chamber consisted of two parts: a 30 cm tall stainless steel bottom collar (length and width of 50 × 50 cm in 2007-2009, and 35 × 35 cm in 2013-2014) and a polyvinyl chloride top chamber (length, width and height of 50 × 50 × 170 cm in 2007-2009, and 35 × 35 × 140 cm in 2013-2014). The bottom collar was inserted into the marsh soils, leaving only 2 cm above the soil surface, approximately 10 days prior to the first flux measurement, and was then left in place for the duration of the study. A fan was installed inside the chamber to mix the headspace air during gas sampling. During each flux measurement, headspace air samples were drawn into air sampling bags (Dalian Delin Gas Packing Co., Ltd., China) at 10-minute intervals over a total duration of 30 min in each sampling plot. The total number of gas samples collected per year was 144 (12 months × 4 time intervals × 3 sites). CH_4 concentrations in the gas samples were determined using a gas chromatograph (GC-2010, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID). The rate of CH_4 emission (mg m^{-2} h^{-1}) was calculated based on the slope of the linear regression between CH_4 concentration in the chamber headspace and time. The annual (cumulative) CH_4 emissions (AE, g CH_4 m^{-2}) (Song et al., 2009; Moore et al., 2011; Xiang et al., 2015) were calculated using Eq. (1):

$$AE = \sum MF_i \times D_i \times 24 \qquad (1)$$

where MF_i is the CH_4 flux at the ith month of the year (mg CH_4 m^{-2} h^{-1}), and D_i is the number of

134 days in the *i*th month of the year.

135 *2.3. Measurement of soil CH_4 production rate*

136 Soil CH_4 production in coastal wetlands has distinct spatio-temporal heterogeneity that
137 could be related to variations in thermal conditions and other abiotic factors (e.g. soil moisture,
138 soil substrate, etc.) (Segers, 1998; Vizza et al., 2017). To assess the variability of soil CH_4
139 production rates across different depths in our marsh, triplicate sediment cores were randomly
140 collected down to a depth of 100 cm in January (winter), March (spring), July (summer), and
141 October (autumn) of 2012. Intact soil cores were collected using a steel sediment sampler (i.d. =
142 5 cm), sub-divided into ten sections at 10 cm intervals in the field, and then kept on ice in coolers
143 and transported to the laboratory within 6 h. The rate of soil CH_4 production was measured
144 following the method of Wachinger et al. (2000). The chambers (5 cm inner diameter, 12 cm
145 height) used for the anoxic incubation of soil cores were made of polyoxymethylene, which was
146 gas-impermeable and inert to CH_4. Before the start of incubation, the chambers were flushed with
147 N_2 gas for 15 min to create an anaerobic condition (Wassmann et al., 1998). The cores were then
148 incubated at *in situ* temperatures, i.e. 10.2, 17.5, 27.5, and 21.5 °C for winter, spring, summer,
149 and autumn, respectively, for a duration of 15 days. We collected 5 mL gas samples from the
150 chamber using a syringe at three day intervals (n = 5) over the course of the incubation, with N_2
151 gas being added after each gas sampling to re-establish the ambient atmospheric pressure. The
152 CH_4 concentrations in gas samples were analysed immediately by gas chromatograph. The CH_4
153 production rates (μg CH_4 g^{-1} (dry weight) day^{-1}) were calculated based on the rate of change in
154 chamber headspace CH_4 concentrations over a 3-day period (Wassmann et al., 1998). The total
155 number of incubations made over the study period was 120 (3 replicates × 4 seasons × 10

depths).

2.4. Porewater collection and analysis of dissolved CH_4 and SO_4^{2-} concentrations

Porewater was sampled using the method of *in situ* dialysis (Ding et al., 2003; Ding et al., 2004a). A series of porewater tubes (5 cm inner diameter) (Ding et al., 2003), with sampling depths of 0–5, 5–10, 10–15, 15–20 and 20–25 cm, were permanently installed adjacent to each CH_4 flux measurement plot, leaving a 5-cm protrusion above the soil surface. The top of each tube was sealed tightly with a cover. Porewater samples were collected in triplicate at each depth interval in January (winter), March (spring), July (summer), and October (autumn) of 2012 and 2013. During each sampling campaign, approximately 10 mL of soil porewater was extracted using a syringe and discarded. Another 10 mL of porewater was then collected and transferred into a 20 mL pre-evacuated vial that was filled with 10 mL of pure N_2 gas (Xiang et al., 2015). About 0.2 mL of $HgCl_2$ solution was further injected into the porewater samples to inhibit bacterial activities without affecting the solubility of CH_4 in water (Butler and Elkins, 1991). The porewater samples were stored at about 4 °C in a cooler and transported immediately to the laboratory within 24 h for analysis. The sample vials were shaken vigorously for 10 min to establish an equilibrium in CH_4 concentrations between the dissolved phase in porewater and the gaseous phase in headspace. The headspace CH_4 concentrations were determined by gas chromatograph, and the dissolved CH_4 concentrations (μmol CH_4 L^{-1}) in porewater were then calculated following the methods of Johnson et al. (1990) and Zhang et al. (2010).

To determine porewater SO_4^{2-} concentrations across different soil depths, another triplicate soil cores were collected down to a depth of 100 cm were collected in January (winter), March (spring), July (summer) and October (autumn) of 2012. The cores were split into ten sub-samples

at 10 cm intervals, which were then immediately sealed in a valve bag, kept on ice in coolers, and transported to the laboratory within 6 h. Upon return to the laboratory, porewater was extracted from the soils at each depth interval by centrifugation at 5000 rpm for 10 min (Cence® L550). The porewater samples were filtered with 0.45 μm acetate fibre membranes, and the SO_4^{2-} concentrations were determined using the barium chromate colorimetric method. The soil SO_4^{2-} concentration data for the 90 and 100 cm depths during the winter were lost due to damage to the incubation chambers.

2.5. Measurement of environmental variables

During each sampling campaign, temperature (°C), pH, and electrical conductivity (EC; mS cm^{-1}) in the top 15 cm soils were measured at each site. Soil temperature and pH were determined *in situ* by using a handheld pH/mV/temperature meter (IQ150, IQ Scientific Instruments, Carlsbad, CA, USA), and soil EC was measured with a EC Meter (2265FS, Spectrum Technologies Inc., Phoenix, AZ, USA). Air temperature (°C) and rainfall were recorded by an automatic meteorological station (LSI-LASTEM, Italy) installed at the Min River Estuary Station of the China Wetland Ecosystem Research Network.

2.6. Data analysis and model formation

Data were log-transformed to approximate normal distributions when selected attributes were skewed. The coefficients of variation (CV) for CH_4 fluxes and environmental variables were calculated by dividing the standard deviation by the mean to determine the magnitude of interannual (among the 5 years) and interseasonal variability (among the 20 seasons observed) (Musavi et al., 2017). Two-way analysis of variance (ANOVA) was used to explore whether seasonality, soil depths or their interaction have fixed effects on soil CH_4 production rates or

porewater CH_4 concentrations, with soil sulfate (SO_4^{2-}) concentration being a covariate.

We recognised that the above formed statistical models in this study might not fit the assumptions of ANOVA, rendering the formal inference based on the p-value of ANOVA potentially unreliable. Apart from the ANOVA models, different mixed-effect models were also used to investigate how soil depths were related to soil CH_4 production rates or porewater CH_4 concentrations, because it would be more feasible to model the variance structure of soil depths in the mixed-effect model framework than ANOVA. Since the different models for soil depths were not nested, likelihood ratio tests could not be used, and the Akaike information criterion (AIC) was used instead for model comparison.

To take into account the possible spatial autocorrelations of soil CH_4 production rates or porewater CH_4 concentrations down the soil profile, we also considered soil depth as a random effect variable in the linear mixed-effect model using the lme function in the nlme package of R (Pinheiro et al., 2017). Our results showed that the AIC values of models fitting soil depth as a fixed factor for both soil CH_4 production rates and porewater CH_4 concentrations (158 and 55, respectively) were significantly lower than those fitting soil depth as a random effect variable (211 and 90, respectively). Hence, we only presented results obtained from the former models fitting soil depth as a fixed factor, which performed slightly better than the mixed linear model.

Linear mixed-effect models were also used to test for differences in interseasonal variability of CH_4 fluxes within sites after accounting for the possible effects of air temperature, soil temperature, precipitation, soil pH and water salinity, with sampling year being fitted as a random intercept to account for the repeated measures of other factors, i.e. interseasonal variability ~ air temperature + soil temperature + precipitation + soil pH + salinity, random = ~ 1 | Year. Similarly,

linear mixed-effect models were used to test for the possible predictors of the variations in CH_4 flux, with the sampling site being selected as a random effect variable to account for the repeated measures in spatial CH_4 flux i.e., CH_4 flux ~ air temperature + soil temperature + precipitation + soil pH + salinity, random = ~ 1 | site. In order to test for temporal autocorrelation, we plotted autocorrelation function (ACF) and partial autocorrelation (PACF) plots of the residuals to help interpretation of the CH_4 flux data. Following Bader et al. (2013), we refitted a model including an autocorrelation function with a first-order autoregressive correlation structure (AR1), specified as "correlation = corAR1 (form=~ date | site)", to account for the repeated measures on 60 different days at the three sites to model the violation of independence of residuals from different sampling days. Significant difference between models with and without AR1 was tested by the anova function in R, and the model with AR1 that showed a significantly lower AIC value was chosen. A variable selection with the fitted global models based on the AIC algorithm and a relative importance method were then used to quantify the contributions of the best predictors (the significant variables of the final model) of the variations in CH_4 flux and their interseasonal variability. For the model selection, we used the stepAIC() function in the R package "MASS", accompanied by the calc.relimp() function with Lindeman-Merenda-Gold (LMG) relative importance method in the R package "relaimpo" (Musavi et al., 2017). The model with the lowest AIC value was chosen, and the relationship between the dependent variables and chosen predictors was further tested by Type II Wald's test implemented in the R package "car".

Besides, the differences in the mean values of environmental variables (precipitation, temperature, soil pH, and soil EC) over the five years were also examined by repeated measures analysis of variance (RMANOVA). For the dataset of each individual year, Pearson correlation

244 analysis was used to examine the relationships (1) between environmental variables and CH_4
245 emissions, soil CH_4 production rates, or porewater CH_4 concentrations, and (2) between CH_4
246 emissions and soil CH_4 production rates or porewater CH_4 concentrations. The interseasonal
247 variability (ISV) of salinity and CH_4 fluxes was determined by dividing the standard deviation of
248 the variables measured at triplicate sampling sites by the average value obtained in each
249 individual season. Temperature sensitivity (Q_{10} value) of CH_4 emissions was calculated following
250 the exponential regression model described by Tong et al. (2015) and Wang et al. (2015). All
251 statistical analyses were performed using R version 3.4.1 (R Development Core Team 2017) and
252 a P value of < 0.05 was considered statistically significant for multiple comparisons. All data
253 were reported as mean ± 1 standard error (SE). All statistical graphs were generated using
254 OriginPro 7.5 (OriginLab Corp. USA).

255 ## 3. Results

256 *3.1. Temporal variations in environmental variables*

257 Figure 2 shows the temporal variations in soil temperature, pH, and EC, all of which showed
258 similar patterns over time for the majority of the study periods. Considerably higher air and soil
259 temperatures and lower EC were observed between May and September than in other months
260 (Fig. 2). Soil pH showed no clear seasonal patterns but varied slightly among measurement
261 events. Over the five-year period, the mean annual air temperatures were very close to the
262 historical average of 22.2 °C (Table 1) while the monthly mean air temperatures followed the
263 long-term historical patterns, with July and August usually being the warmest months and
264 January and February the coldest (Fig. 2). Fig. S1 shows the monthly precipitation amounts over
265 the five study years, which varied significantly both seasonally and inter-annually. Nearly half of

266 the annual rainfall occurred in summer, with several heavy rainfall events in July and August.
267 Significantly higher annual precipitation was observed in 2013, while lower precipitation
268 occurred in 2007 and 2009.

269 *3.2. Dynamics of soil CH_4 production rates and porewater CH_4 concentrations*

270 The range of average soil CH_4 production rates across all depths among the four seasons was
271 large, spanning three orders of magnitude from 0.04 to 1.67 μg CH_4 g^{-1} day^{-1}. Soil CH_4
272 production rates varied significantly with season and soil depth ($P < 0.05$) (Table 2 and Fig. 3).
273 The highest and lowest soil CH_4 production rates were observed in the summer and winter,
274 respectively (Table 2 and Fig. 3). Significantly higher CH_4 pro\duction rates were observed from
275 the topsoil (5–15 cm) as compared to other soil depths during the spring, summer, and autumn (P
276 < 0.05) (Table 2 and Fig. 3), indicating a decreasing trend with depth. There were also significant
277 interactions between seasons and soil depths in affecting soil CH_4 production rates ($P < 0.05$)
278 (Table 2).

279 Fig. 4 shows the seasonal variations in dissolved CH_4 concentrations down the soil profile.
280 Porewater CH_4 concentrations varied significantly with both depth and season ($P < 0.01$) (Table
281 2), ranging between 2 and 457 μmol L^{-1} (Fig. 3). We observed a significant, increasing trend of
282 porewater CH_4 concentrations with depths (Table 2 and Fig. 4), and substantially higher CH_4
283 concentrations at all depths during the summer ($P < 0.01$) (Table 2 and Fig. 4).

284 *3.3. Temporal variations in CH_4 emissions*

285 *3.3.1. Seasonal variations in CH_4 emissions*

286 Across all years, the highest CH_4 emissions were observed between April and October (Fig.
287 5). Fluxes were generally low between November and March, except in 2013 in which the peak

of CH_4 emission occurred in December and January. When averaging the monthly fluxes over five years, a strong seasonal pattern in CH_4 emissions emerged, with generally low values in spring, a maximum in summer, and a minimum in winter (Fig. 6). Meanwhile, we observed considerable variations in both mean CH_4 fluxes (Table 1) and the timing of maximum emissions (Fig. 5) among different years. For example, the maximum CH_4 emissions occurred in May–June in 2013, but in August–October in 2014. Clear peaks of CH_4 emissions were not observed in 2007 and 2009, with only slightly higher fluxes being detected between April and October. Salinity was the most important factor governing the seasonal variability of CH_4 emissions (Table 3), with a significant positive correlation observed between the two (Fig. 7).

3.3.2. Inter-annual variations in CH_4 emissions

The coefficient of variation of annual mean CH_4 emissions over the five years was 67%, which implied a considerable inter-annual variability. Over the study period, the mean annual CH_4 emissions from the *C. malaccensis* marsh ranged between 0.71 and 5.10 mg CH_4 m^{-2} h^{-1}, leading to annual cumulative emissions of 6.2-48.9 g CH_4 m^{-2} (Fig. 5 and Table 1). Significantly lower and higher CH_4 effluxes were observed in 2007 and 2013, respectively, as compared to other years (Table 1). According to the AIC-based model selection, variations in CH_4 emissions were best predicted by soil temperature, precipitation and salinity (represented by EC) (Table 3), which independently explained 60.0% (positive effect), 21.7% (positive effect) and 18.2% (negative effect) of the variations, respectively (Fig. 7).

4. Discussion

4.1. Variability of soil CH_4 production rates and porewater CH_4 concentrations

Soil CH_4 production rates from our estuarine marsh demonstrated significant variations

down the soil profile (Table 2 and Fig. 3), with the highest rates occurring in the top soil layer (5–15 cm depth) in all seasons except winter, which was in accordance with the results of previous studies (van den Pol-van Dasselaar & Oenema, 1999; Liu et al., 2011; Knoblauch et al., 2015). We found a negative correlation between soil CH_4 production rates and porewater SO_4^{2-} concentrations along the soil profile (Fig. S2). The higher porewater SO_4^{2-} concentrations in the deeper soil layer can help the sulfate-reducing bacteria in outcompeting the methanogens for substrates, thereby inhibiting CH_4 production at depth (van der Gon et al., 2001; Purdy et al., 2003; Vizza et al., 2017). The vertical distribution of CH_4 production rates down the soil profile might also be related to the differences in substrate quantity and quality. Previous studies in wetlands have shown that soil CH_4 production rate increased with the availability of labile carbon fractions (Updegraff et al., 1995; Liu et al., 2011; Inglett et al., 2012). A previous study conducted at our site has shown that the majority of *C. malaccensis* root biomass was distributed in the upper surface layer (Tong et al., 2011), which could provide an abundant supply of labile carbon to support the metabolic activity of methanogens (Ström et al., 2012). On the other hand, we found a significant increase in porewater CH_4 concentrations with depth, which was opposite to the pattern of CH_4 production rates in the soil profile (Table 2 and Fig. 4). The concentration of CH_4 in porewater is influenced by both CH_4 production and loss. In spite of a high CH_4 production rate in the top soils, we hypothesize that the lower porewater CH_4 concentration observed could be related to the tidal actions, which are one of the key physical processes shaping the biogeochemical processes in coastal wetlands (Tong et al., 2010). The top soil layers were subjected to frequent tidal flushing, which could enhance CH_4 export to the tidal waters and reduce the accumulation of CH_4 in porewater (Lee et al., 2008). In addition, the inflow of tidal

water would bring along a large amount of oxygen and SO_4^{2-} to the surface soils, thereby increasing the soil redox potential and promoting methanotrophy in the upper layers (Ding et al., 2003; Sun et al., 2013).

We observed distinct seasonal variations in soil CH_4 production rates with significantly higher values in the summer (Table 2 and Fig. 3), which were in accordance with the findings of previous studies (Bergman et al., 2000; Avery et al., 2003; Tong et al., 2012). Similarly, porewater CH_4 concentrations were found to be significantly higher during the summer season. It is generally acknowledged that CH_4 production rates would vary seasonally as a function of temperature (e.g. Segers, 1998; Inglett et al., 2011). In our study, soil temperature had an exponential relationship with soil CH_4 production rates (Fig. S3), and positive correlation with porewater CH_4 concentrations ($r = 0.662$, $p < 0.01$, $n = 24$), pointing to the positive impacts of temperature on microbial-mediated methanogenesis. Moreover, the amount of plant biomass in this wetland was found to be much higher in summer than in winter (Tong et al., 2011). The enhanced plant productivity and subsequently supply of labile carbon substrates through root exudation in the summer period would likely play a role in stimulating methanogenic activities (Whiting & Chanton, 1993; Bergman et al., 2000; Walter et al., 2001) and hence increasing the concentrations of CH_4 in soil porewater (Xiang et al., 2015). In addition, the increased freshwater discharge from the estuary in summer time provided a dilution effect that significantly reduced the salinity of tidal water, which would in part facilitate methanogenesis through reduced competition with sulfate-reducing bacteria (Sinke et al., 1992). We observed a significant and negative correlation between salinity and porewater CH_4 concentration ($r = -0.653$, $p < 0.01$, $n = 24$) that supported this hypothesis.

354 *4.2. Temporal variations of CH_4 emissions*

355 *4.2.1. Seasonal variability*

356 In this study, CH_4 emissions from the subtropical estuarine marsh varied considerably
357 among different seasons. The seasonal mean CH_4 emissions over the five-year period were
358 correlated significantly with both soil CH_4 production rates (0–20 cm depth) (Fig. S4) and
359 porewater CH_4 concentrations (Fig. S5). As such, the seasonal pattern of CH_4 emission (Fig. 6)
360 was highly similar to that of soil CH_4 production rates (Fig. 3) and porewater CH_4 concentrations
361 (Fig. 4). This strong relationship was expected since a high CH_4 production rate in soils would
362 increase the supply of CH_4 to soil porewater, and subsequently enhance net CH_4 emissions to the
363 atmosphere owing to the steeper concentration gradient.

364 The seasonal variability of CH_4 emissions could be governed by the interactions of a number
365 of environmental variables. Our results showed that salinity was one dominant factor controlling
366 the variations of CH_4 flux among different seasons (Table 3 and Fig. 7b). Numerous studies have
367 reported a significant reduction in CH_4 emissions from coastal wetlands with salinity (Bartlett et
368 al., 1987; Magenheimer et al., 1996; Poffenbarger et al., 2011; Tong et al., 2012; Sun et al., 2013;
369 Vizza et al., 2017). The significantly lower soil salinity (represented by EC) observed between
370 May and September in our site would significantly hinder methanogenic activities owing to the
371 presence of alternate electron acceptors (Welti et al., 2017). Salinity could also affect CH_4
372 production through its effects on extracellular enzyme activities and carbon mineralization rates
373 (Chambers et al., 2013; Neubauer et al., 2013). Meanwhile, salinity might also affect
374 methanotrophic activities directly or indirectly, which in turn alter the rate of CH_4 emissions from
375 wetlands. Only few studies have thus far directly examined the mechanistic processes, i.e. CH_4

376 production and oxidation, involved in the suppression of net CH_4 flux by salinity (Vizza et al.,
377 2017). Further studies should be carried out to explore the exact impacts of salinity on various
378 biogeochemical processes in soils in affecting CH_4 dynamics.

379 Temperature was another important driver of the changes in CH_4 emissions from our *C.*
380 *malaccensis* marsh, as shown by the strong relationships observed between air/soil temperature
381 and CH_4 flux in individual years (Table 4). An increase in temperature could enhance CH_4
382 emissions by increasing methanogenic activities, stimulating root exudations (Song et al., 2009;
383 Yvon-Durocher et al., 2014; Olsson et al., 2015), as well as facilitating plant-mediated CH_4
384 transport (Hosono and Nouchi, 1997). Meanwhile, we found that the temperature sensitivity of
385 CH_4 flux varied considerably among different years over the study period, with Q_{air10} and Q_{soil10}
386 values ranging from 2.46 to 5.30, and from 3.66 to 7.92, respectively (Fig. S6). Our results
387 suggest that the estimation of long-term (multi-year) CH_4 emissions based on simple
388 extrapolations of the relationships between temperature and CH_4 flux derived from short-term (<
389 1 year) measurements might not be reliable and introduce significant biases. Apart from
390 temperature, the hydrologic conditions of the site could also affect CH_4 emissions by controlling
391 the depths of the oxic and anoxic layers as well as soil redox potential (Dinsmore et al., 2009).
392 The disproportionately high amount of precipitation received during the summer (Fig. S1) could
393 favour the formation of a wetter and more anaerobic environment in the soils for methanogenesis
394 (Lai et al., 2014). Furthermore, the total amount of plant biomass (aboveground + belowground)
395 at our marsh site was found to vary significantly among seasons in the following order: summer >
396 autumn > spring > winter (Tong et al., 2011), which could exert influences on the variability of
397 plant-mediated CH_4 emissions via primary production and substrate supply.

Based on our five-year data set, we observed that peak CH_4 emissions generally occurred during the summer period when temperature was high and conductivity was low, which favored methanogenesis. Yet, the exact timing of peak CH_4 emissions varied from one year to another that could be partly related to the inter-annual variations in the timing of maximum monthly precipitation, which governed the extent of anaerobic conditions in soils. For instance, the timing of peak CH_4 emission coincided with that of maximum monthly precipitation in 2008 and 2014, which happened to be in the months of July and August, respectively. Yet, in 2013, the extremely high precipitation amount in July implied a lack of abundant sunlight during this period, which could hinder photosynthesis by marsh plants and the supply of labile carbon from photosynthates to soils for methanogenesis. Our results point to a need of carrying out more in-depth studies in future to disentangle the specific influences of various environmental factors on the seasonal variability of CH_4 emission in coastal marshes.

4.2.2. Inter-annual variability

In the present study, CH_4 emissions from the brackish *Cyperus malaccensis* marsh showed substantial inter-annual variability (Fig. 5 and Table 3). Previous studies have shown that the inter-annual variability of CH_4 emissions was governed by water table position (Moore et al., 2011), peat temperature (Shannon and White, 1994; Lai et al., 2014), and precipitation (Song et al., 2009). According to the AIC-based model selection, we found that soil temperature and salinity were the primary determinants of the inter-annual variability of CH_4 flux at our site (Table 3 and Fig. 7a). The effects of soil temperature and salinity could be related to the production of substrate precursors and methanogenic activity as described previously (Whalen et al., 2005; Dinsmore et al., 2009; Lai et al., 2014; Yvon-Durocher et al., 2014). In addition, we

found strong correlations between CH_4 flux and precipitation amount over the study period (Table 4). Among the five study years, the lowest annual mean precipitation was recorded in 2007, which was significantly lower than that in 2008, 2013 and 2014 (1362 vs. 1485-1890 mm, p <0.05, Fig. S1). It was likely that the much lower CH_4 emission observed in 2007 was at least partly related to the significantly lower amount of precipitation received in this particularly dry year, which provided a more aerobic and less favorable environment for methanogenesis to take place. Other factors, such as primary productivity and water table depth, might also contribute to the inter-annual variations in CH_4 flux and deserve further investigations.

It is noteworthy that significantly higher CH_4 emissions were observed in 2013 and 2014 as compared to other years ($p < 0.05$; Table 1), which could be related to the increased discharge of nutrient-enriched effluents from the aquaculture ponds in the surrounding region. Starting from 2011, the conversion of natural tidal marshes into aquaculture ponds has become increasingly dominant in the Shanyutan wetland. A previous study estimated that about 29% of total nitrogen and 16% of total phosphorus added to the ponds in the form of feeds and fertilizers were actually assimilated by fish and shrimps during a production cycle (Avnimelech and Ritvo, 2003). The majority of added nitrogen and phosphorus would then eventually be discharged as effluents that are rich in particulate matters (e.g. uneaten feeds, faeces, phytoplankton) and dissolved nutrients (Jackson et al., 2004; Molnar et al., 2013) into the adjacent waterbodies, further stimulating microbially-mediated CH_4 emissions. Studies based on both laboratory incubations and field measurements have demonstrated the positive effects of exogenous nutrient loading on CH_4 production and emissions (Liu and Greaver, 2009; Chen et al., 2010). Hu et al. (2017) also reported that the addition of nitrogen strongly stimulated CH_4 emissions from the *C. malaccensis*

442 marsh in our study area.

443 *4.3. Implications and further outlook*

444 It is generally considered that coastal wetlands have a lower CH_4 emission rate than other
445 natural wetlands owing to the inhibitory effect of high SO_4^{2-} concentrations (Bartlett et al., 1987;
446 Ding et al., 2004b; Saarnio et al., 2009; Poffenbarger et al., 2011). However, we found that the
447 mean annual CH_4 emission from our subtropical brackish marsh in the Min River Estuary was
448 23.8±18.1 g CH_4 m^{-2} yr^{-1}, which was 1.8 times higher than the average of 13.3 g CH_4 m^{-2} yr^{-1}
449 across China's natural wetlands (Wei and Wang, 2017), and also substantially higher than the
450 annual CH_4 flux reported in the Atlantic blanket bogs (5.5–6.2 g CH_4 m^{-2} yr^{-1}) (Laine et al., 2007;
451 Koehler et al., 2011), Finnish bogs (5.2–6.8 g CH_4 m^{-2} yr^{-1}) (Alm et al., 1999), as well as Swedish
452 and Minnesota fens (12.0–19.5 g CH_4 m^{-2} yr^{-1}) (Shurpali et al., 1993; Rinne et al., 2007; Nilsson
453 et al., 2008). The range of CH_4 emissions from our brackish marsh (6.19–48.86 g CH_4 m^{-2} yr^{-1})
454 was also comparable to that from the coastal wetlands in South India (17.3–118.4 g CH_4 m^{-2} yr^{-1})
455 (Purvaja and Ramesh, 2001), freshwater marshes in Europe (10–90 g CH_4 m^{-2} yr^{-1}) (Saarnio et al.,
456 2009), and freshwater marshes in China (4.9–94.1 g CH_4 m^{-2} yr^{-1}) (Ding et al., 2004b). Our
457 results suggest that subtropical estuarine brackish marshes could be important sources of
458 atmospheric CH_4 and thus should not be overlooked in greenhouse gas accounting for their
459 contributions to global climate change.

460 Numerous efforts have been made to estimate regional CH_4 emissions from coastal wetlands
461 by extrapolating short-term (< 2 year) field measurements to longer periods in a given area (e.g.
462 Purvaja and Ramesh, 2001; Ding et al., 2004b; Saarnio et al., 2009; Ortiz-Llorente and
463 Alvarez-Cobelas, 2012). However, our results show that CH_4 emissions from the estuarine

marshes had strong inter-annual variability, which suggest a high uncertainty of regional estimates of CH_4 emissions relying only on short-term measurements. It is of paramount importance to take into account long-term observations in order to reduce the uncertainty of CH_4 flux estimations and improve our understanding of the impacts of wetlands on the atmospheric CH_4 balance. Moreover, most of the existing process-based models used for estimating CH_4 flux from coastal wetlands fail to consider the influence of salinity (Li et al., 2016). In this study, we observed a significant and strong relationship between salinity and CH_4 emissions (Table 3 and Table 4), indicating that the interactions between temperature, salinity, and other biotic/abiotic factors should be addressed comprehensively to improve the current CH_4 flux models.

It should be noted that the findings of this study were limited by several uncertainties, which could be associated with the following aspects: (1) single time-point measurements do not fully capture the episodic and high magnitude events of CH_4 release; (2) a limited number of flux measurement sites results in a lack of adequate spatial representation; (3) chamber measurement problems, such as changes in temperature; and (4) the lack of CH_4 emission observations during flood tide. Future research programs will thus need to increase the frequency of sampling *in situ* for longer periods and at different spatial scales as well as include innovative techniques (e.g. eddy covariance tower) in order to measure CH_4 releases from coastal marshes.

5. Conclusions

Our long-term measurements of CH_4 emissions from the subtropical estuarine brackish *C. malaccensis* marsh ecosystem in the Min River Estuary, southeast China over five years revealed strong inter-annual and seasonal variabilities of CH_4 fluxes. The temporal variations in CH_4 emissions from our marsh were mainly related to the variations in soil temperature and salinity,

while the potential roles of primary productivity and precipitation should not be overlooked. Our results suggest that long-term, high-frequency observation of CH_4 emissions is essential for making reliable flux estimates from the coastal marshes. In addition, the significant relationships among soil CH_4 production rates, porewater CH_4 concentrations, and net CH_4 emissions observed in this study highlight the great potential in successfully simulating these processes using biogeochemical models once the influences of key environmental factors are properly quantified.

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