# Methane Emissions from Estuarine Coastal Wetlands: Implications for Global Change Effect

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Global warming, rising sea levels, and saltwater intrusion interact to affect carbon cycling, specifically methane ($CH_4$) flux in estuarine coastal wetlands. In the present study, *Phragmites australis* and *Spartina alterniflora* communities in the intertidal zone of the Yangtze estuary were selected for investigating $CH_4$ flux under different temperature (natural/warming), water level (high/low), and salinity (0, 5, 15, and 30‰) conditions. The average $CH_4$ flux (from April to October 2016) under natural conditions was $141.0 \pm 21.5$ and $502.8 \pm 65.3$ μmol m$^{-2}$ h$^{-1}$ for *P. australis* and *S. alterniflora* communities, respectively. Warming had a particularly pronounced effect on $CH_4$ emissions from the *P. australis* community and increased $CH_4$ flux by 130%. There was a significant correlation between $CH_4$ flux and water level; at high water levels, $CH_4$ flux was 2.64- and 3.78-fold higher in *P. australis* and *S. alterniflora* communities, respectively. Salinity had a significant pronounced effect on $CH_4$ emissions from the *P. australis* community, and there was a clear order (5‰ > 15‰ > 0‰ > 30‰) in $CH_4$ flux. The interaction between temperature and water level was the most important factor controlling $CH_4$ flux from wetlands; $CH_4$ emissions were greater at higher temperature and higher water levels. However, at low water level, the effect of salinity was more prominent. The results suggest that $CH_4$ flux from estuarine wetlands could be further enhanced in the future under the influence of rising sea levels due to global warming.

**Core Ideas**

- We investigated estuarine environmental factors on $CH_4$ flux from two vegetation communities.

- Warming significantly affected on $CH_4$ emission from the *Phragmites australis* community.

- There was a significant correlation between $CH_4$ flux and water level in both communities.

- Salinity significantly affects $CH_4$ emissions from the *Phragmites australis* community.

- The interaction between temperature and water level was the most important factor controlling $CH_4$ flux.

Methane ($CH_4$) is one of the most important greenhouse gases and plays a significant role in the atmosphere. The concentration of $CH_4$ has increased by 157% since the industrial revolution (WMO, 2018). Natural wetlands are an important sink for atmospheric $CH_4$, and emission processes and inventories have received extensive attention (Saunois et al., 2016; Wang et al., 2009). Sea level rise caused by global warming, accompanied by the intensification of saltwater intrusion in coastal wetlands, affects the bio-

geochemical cycles of tidal freshwater and brackish ecosystems (Chambers et al., 2013).

According to the Intergovernmental Panel on Climate Change report, global temperature will rise further. Compared with 1986 to 2005, the average global surface temperature from 2016 to 2035 is expected to rise by 0.3 to ∼0.7°C and by the end of this century will rise by 0.3 to ∼4.8°C (IPCC, 2014). Methanogenesis is temperature sensitive, and the rate of $CH_4$ production is linearly or exponentially correlated with temperature (Wang et al., 2009). The metabolic rate and substrate supply of methanogenic microorganisms, both affected by temperature, are important factors controlling $CH_4$ emissions from different types of wetland ecosystems (Whalen, 2005). Drake et al. (1996) reported that faster growth of wetland vegetation is accelerated at higher temperatures, stimulating the production of more organic matter, which alters the type and quantity of methanogenic substrates, indirectly affecting the function of methanogens. In addition, $CH_4$ diffusion velocity is promoted by increasing temperature (Nahlik and Mitsch, 2011).

Global sea levels have risen on average 0.17 m due to anthropogenic climate change in the 20th century (Church and White, 2011). By 2100, global mean sea level will increase between 0.26 and 0.55 m and 0.45 and 0.82 m under the lowest and highest proposed greenhouse gas concentration scenarios, respectively (IPCC, 2014). Methane is produced under strict anaerobic conditions. Rising sea levels exert a powerful influence on $CH_4$ emissions, extending the immersion time of estuarine wetland habitats, blocking $O_2$ from diffusing into deeper soils, and thereby determining the depth of the aerobic anoxic boundary and the redox level (Shoemaker et al., 2012). Therefore, the soil moisture status largely determines $CH_4$ emissions in a given region. Additionally, standing water depth determines the type of marsh plants, which governed $CH_4$ transport, and the amount of plant litters, which resulted in the difference in labile organic C for methanogenesis among marshes. Labile organic C greatly affects $CH_4$ concentration and the total amount of $CH_4$ in porewater, whereas the latter is significantly related with $CH_4$ emissions (Ding et al., 2002).

Rapid sea level rise, together with storm surges, droughts, and extreme tide events, may increase the frequency of seawater intrusions farther into historically low-salinity estuary zones (Neubauer et al., 2013). The increase of soil salinity, especially NaCl ($Cl^-$), can result in a high-ionic-stress environment. High ionic strength can further induce osmotic stress in microbes, interfering with their cellular functions and reproduction or even causing cell lysis and the divergence of microbial community composition (Ikenaga et al., 2010). After salinity intrusion, cations in seawater (e.g., $Na^+$, $Mg^{2+}$, $Ca^{2+}$, and $K^+$) may quickly replace cations adsorbed in tidal wetland sediments (e.g., $NH_4^+$, $Fe^{2+}$, and $Al^{3+}$) and then release them into porewater, which will eventually alter the pathways of microbial organic matter mineralization (Weston et al., 2006). Specifically, microbial pathways of organic matter mineralization shifted from methanogenesis to iron reduction and then followed by a transition to sulfate reduction, which became the dominant (>95%) organic matter miner-

alization pathway (Weston et al., 2006). Furthermore, it is generally acknowledged that saltwater intrusion will introduce high concentrations of sulfate ($SO_4^{2-}$) (Chambers et al., 2013). The presence of $SO_4^{2-}$ in marsh soils is conducive to allowing sulfate-reducing bacteria to outcompete methanogens for energy sources, and the enhanced $HS^-$ concentrations, a byproduct of microbial sulfate reduction, can also exert ionic toxicity on methanogens (Joye and Hollibaugh, 1995), consequently inhibiting $CH_4$ production. In addition, sulfate-reducing bacteria can oxidize $CH_4$ (Capone and Kiene, 1988), directly reducing $CH_4$ production.

The Yangtze has one of the biggest estuaries in the world, with extensive tidal wetlands. This is of special concern in the Yangtze where estuarine environmental factors are combined with past and current changes, such as soil microbial respiration and carbon (C) degradation, and affect the C cycles of microbial communities (Hu et al., 2014). In the present study, we selected two typical intertidal vegetation communities in the Yangtze estuary, based on *Phragmites australis* and *Spartina alterniflora*, as model mesocosms to investigate the effects of three environmental factors (warming, rising sea levels, and saltwater intrusion). The goal of this study was to expose tidal flat sediments to different estuarine environmental factors in mesocosms that simulated conditions and quantify the effect on methanogenesis ($CH_4$ flux) from different vegetation communities of estuarine wetlands in the Yangtze and to contribute to research on greenhouse gas emissions and the responses of tidal wetlands to changes of estuarine environmental factors.

## MATERIALS AND METHODS
### Study Area

Chongming Dongtan, in the eastern part of Chongming Island in the Yangtze estuary, was selected as the sampling area, and a typical *P. australis* community has developed in the upper part of the intertidal zone. Since 1979, *S. alterniflora* has been introduced to promote siltation and to control tidal flat erosion, and this species has rapidly expanded following establishment. At present, the intertidal vegetation wetland community is dominated by *P. australis* and *S. alterniflora* (Li and Zhang, 2006).

### Establishing Mesocosms

Sediment samples (length, 40.0 cm; width, 30.0 cm; depth, 50.0 cm), including roots and shoots, were collected from typical *P. australis* and *S. alterniflora* communities outside the Chongming Dongtan levee (121°59′22″ E; 31°30′35″ N) in the winter of 2016. The internal size of the incubator (length, 38.0 cm; width, 30.0 cm; depth, 50.0 cm) matched the sediment boxes, and there was a U-shaped stainless-steel groove (height, 3.0 cm; width, 2.0 cm) at the top of the incubator. A total of 48 sediment boxes from *P. australis* and 48 sediment boxes from *S. alterniflora* communities were collected, transported to the laboratory, and placed in incubators. Mesocosms consisted of two parts: a natural room (simulating the natural temperature conditions) and an artificial control room (simulating the warming conditions). For natural conditions, sediment boxes were

placed directly on the ground, and a transparent film was used to block the rain. The artificial control room was a glass house in which rising temperatures could be simulated. In each room, the simulation environment included two water levels (high and low). In addition, according to the tidal wetlands along an estuarine salinity gradient (Luo et al., 2017), there were four salinities (0, 5, 15, and 30‰). The atmospheric temperature simulation in the artificial control room mainly relied on the greenhouse effect and the air conditioning system and was 2.5 to 10.5°C higher than the natural room. We periodically added corresponding artificial seawater to the vegetation incubators to maintain the level in the high-water-level group at 3.0 cm above the sediment interface. The level in the low-water-level group was the same as the sediment interface height. In addition, to simulate tidal movements, the water in the container was drained through a hole at the bottom of the incubator and refilled to the original water level after 48 h. In each room, eight different culture conditions (high + 0‰/low + 0‰/high + 5‰/low + 5‰/high + 15‰/low + 15‰/high + 30‰/low + 30‰) were set according to different water levels and salinity to carry out the cultivation of *P. australis* and *S. alterniflora* communities, respectively, and three parallel samples were set for each condition. In each room, there were 24 *P. australis* and 24 *S. alterniflora* community incubators (Supplemental Fig. S1A, B).

## Simulation Procedure

The static closed chamber technique was used to measure $CH_4$ flux. The sampling box (length, 38.0 cm; width, 30.0 cm; height, 100.0 cm) has a closable top and an open bottom. The bottom of the box was directly inserted into the U-shaped stainless steel groove at the top of the incubators (Supplemental Fig. S1C). A rubber tube (length, 20.0 cm; inner diameter, 0.5 cm) was mounted on the top of the sampling box, and the end of the tube was sealed with a medical three-way valve. All rubber hoses and joints were sealed with silicone to ensure air tightness during sampling. Samples were taken monthly from April to October 2016. Gas samples were collected at 9:00 AM (0900 h), 12:00 PM (1200 h), 3:00 PM (1500 h), and 6:00 PM (1800 h) for 30.0 min each time. The boxes were placed in the U-shaped stainless steel grooves and sealed with an appropriate amount of water. A 50-mL sample of gas was extracted with a syringe at 0, 10, 20, and 30 min each time and injected into the sampling bag.

While sampling, the temperature was measured using an anemometer (Kestrel-4000, Kestrel). Photosynthetically active radiation was measured using the TES-1332 photometer (TES). The number of plants and the plant height were measured at the site. The aboveground parts of plants were removed and bagged, and the wet biomass was measured in the laboratory.

## Sample Analysis

The bulk density was determined after oven drying a known volume of subsample at 70°C until constant weight in the field. At the end of the experiment, soils were recovered from each box and analyzed for field water content (based on the mass loss before and after drying at 105°C) and organic matter content (loss on ignition, 5 h at 550°C). Soil pH was measured with the portable water quality multiparameter detector (HACH). A 2:1 (water/soil) suspension was created and allowed to equilibrate for 30 min before measurement (Thomas, 1996). The available phosphorus content in soil was extracted with sodium bicarbonate solution supplemented with with molybdenum antimony anti-reagent and determined by colorimetric method (Olsen, 1954). The available nitrogen content in the soil was measured with NaOH-hydrolyzation diffusion methods (Stanford, 1982). The available potassium content in soil was extracted by ammonium acetate and determined by flame photometry (Carter, 1993). The $CH_4$ content of gas samples was analyzed using a gas chromatograph (7890A, Agilent) equipped with a flame ionization detector and a column (2 m × 2 mm) packed with XMS (60/80). The flame ionization detector temperature was 200°C, the column temperature was 55°C, and high-purity $N_2$ was used as the carrier gas (30 cm$^3$ min$^{-1}$).

## Data Calculation

Methane flux was calculated using the box gas flux formula:

$$F = (\Delta c/\Delta t) \times V/A$$

where *F* is the gas discharge flux (μmol $CH_4$ m$^{-2}$ h$^{-1}$), *V* is the volume of the enclosure (L), *A* is the cross-sectional area of the enclosure (m$^2$), and $\Delta c/\Delta t$ is the change in the concentration of $CH_4$ over time (μmol m$^{-2}$ h$^{-1}$).

## Data Analysis

All graphs were drawn using OriginLab OriginPro9.0 and Golden Software Surfer14.0. IBM SPSS20 was used for analysis of data correlations and difference testing. The differences among conditions were analyzed using one-way ANOVA with Tukey's HSD for post hoc comparisons. The paired sample *t* test was used to test whether the environmental factors have a significant impact on each treatment. Error bars in the graphs represent the SD of parallel sample data. All analyses used a significance factor of α = 0.05.

## RESULTS
### Sediment Parameters and Vegetation Growth Parameters

The average bulk density of sediment samples in the *P. australis* community (1.17 ± 0.06 g cm$^{-3}$) was higher than that of the *S. alterniflora* community (0.87 ± 0.06 g cm$^{-3}$) at the beginning of the cultivation. There were no significant differences in sediment parameters (pH, C%, nitrogen content, phosphorus content, and potassium content) in the two communities between the beginning and the end of the cultivation ($p > 0.05$), and there were no correlations between sediment parameters and $CH_4$ emissions from the two communities ($p > 0.05$). The growth of *P. australis* was mainly concentrated from April to June and proceeded gradually thereafter, whereas the growth of *S. alterniflora* continued until August. The density and biomass of the

**Table 1. Sediment parameter and vegetation growth parameter values at the beginning and the end of the cultivation. Data presented according to temperature treatment (natural and warming, *n* = 24).**

| | *Phragmites australis* | | | *Spartina alterniflora* | | |
|---|---|---|---|---|---|---|
| | **Beginning** | **Nature** | **Warming** | **Beginning** | **Nature** | **Warming** |
| *Sediment parameters* | | | | | | |
| Bulk density, g cm$^{-3}$ | 1.17 ± 0.06† | ND‡ | ND | 0.87 ± 0.06 | ND | ND |
| pH | 8.58 ± 0.10 | 8.62 ± 0.04 | 8.63 ± 0.03 | 8.72 ± 0.10 | 8.74 ± 0.05 | 8.70 ± 0.04 |
| C, % | 1.53 ± 0.03 | 1.47 ± 0.03 | 1.45 ± 0.03 | 1.61 ± 0.14 | 1.59 ± 0.03 | 1.60 ± 0.02 |
| N, mg kg$^{-1}$ | 25.3 ± 0.36 | 23.9 ± 0.59 | 23.7 ± 0.60 | 22.3 ± 1.04 | 22.2 ± 0.47 | 23.2 ± 1.22 |
| P, mg kg$^{-1}$ | 167.2 ± 8.76 | 173.7 ± 4.05 | 177.7 ± 3.62 | 154.8 ± 6.87 | 155.9 ± 3.32 | 161.6 ± 3.40 |
| K, g kg$^{-1}$ | 27.7 ± 1.08 | 28.0 ± 0.64 | 28.1 ± 0.47 | 30.3 ± 0.89 | 32.3 ± 0.67 | 31.1 ± 0.53 |
| *Plant growth parameters* | | | | | | |
| Height, cm | 23.1 ± 3.13 | 136.5 ± 9.02 | 138.3 ± 8.41 | 20.5 ± 0.87 | 121.0 ± 1.38 | 132.0 ± 3.60 |
| Density, shoot m$^{-2}$ | 67.7 ± 14.0 | 113.6 ± 15.4 | 70.5 ± 7.73 | 124.0 ± 9.11 | 179.6 ± 6.38 | 157.3 ± 12.8 |
| Biomass, g m$^{-2}$ | ND | 1239.6 ± 269.8 | 745.6 ± 158.7 | ND | 1327.7 ± 42.5 | 1602.4 ± 383.7 |

† Values are mean ± SE. There were no significant differences in sediment parameters (pH, C%, nitrogen content, phosphorus content and potassium content) in the two communities between the beginning and the end of the cultivation ($p > 0.05$).

‡ No data (the bulk density of the two communities were measured at the beginning, the bulk density of different treatments after cultivation was not measured, or the biomass of the two communities before cultivation was not measured).

two communities continued to increase during the cultivation. In October, the average plant density of *P. australis* (113.6 ± 15.4 shoot m$^{-2}$) and average biomass (1239.6 ± 269.8 g m$^{-2}$) under natural conditions were higher than under warming conditions (density, 70.5 ± 7.73 shoot m$^{-2}$; biomass, 745.6 ± 158.7 g m$^{-2}$). The average density of *S. alterniflora* was also higher under natural conditions (179.6 ± 6.38 shoot m$^{-2}$) than under control conditions (157.3 ± 12.8 shoot m$^{-2}$), but the average biomass was higher under control conditions (1602.4 ± 383.7 g m$^{-2}$) than under natural conditions (1327.7 ± 42.5 g m$^{-2}$). The density and biomass of the *P. australis* community were lower than the *S. alterniflora* community (Table 1).

## Impact of Environmental Factors on CH$_4$ Emissions
### Effect of Temperature

There were significant differences in average CH$_4$ flux between natural and warming conditions in the *P. australis* community (159.7 ± 28.0; 204.4 ± 32.8 µmol m$^{-2}$ h$^{-1}$; $p < 0.05$). In particular, at high water levels and in freshwater (0‰), the average CH$_4$ flux between natural and warming conditions of the *P.*

*australis* community (141.0 ± 47.2; 324.8 ± 87.6 µmol m$^{-2}$ h$^{-1}$) was increased by 2.3-fold in warming conditions. However, at the end of the growing season, the effect of warming was not obvious (Fig. 1). For the *S. alterniflora* community, CH$_4$ flux did not differ significantly between different conditions ($p > 0.05$).

### Effect of Water Level

The CH$_4$ flux differed significantly between the two water levels, with the high water level exhibiting considerably higher average CH$_4$ flux (100.3 ± 21.8, 264.9 ± 32.8 µmol m$^{-2}$ h$^{-1}$ for *P. australis*; 186.7 ± 44.9, 705.7 ± 76.0 µmol m$^{-2}$ h$^{-1}$ for *S. alterniflora*; $p < 0.01$). Regardless of temperature or salinity, the effect was extremely significant ($p < 0.01$). During the entire growing season, the CH$_4$ flux of the *P. australis* community at high water level conditions was 2.64-fold greater than the low-water-level group, and the CH$_4$ flux of the *S. alterniflora* community was more sensitive to water level changes, with a 3.78-fold difference between the high and low water level groups (Fig. 2).
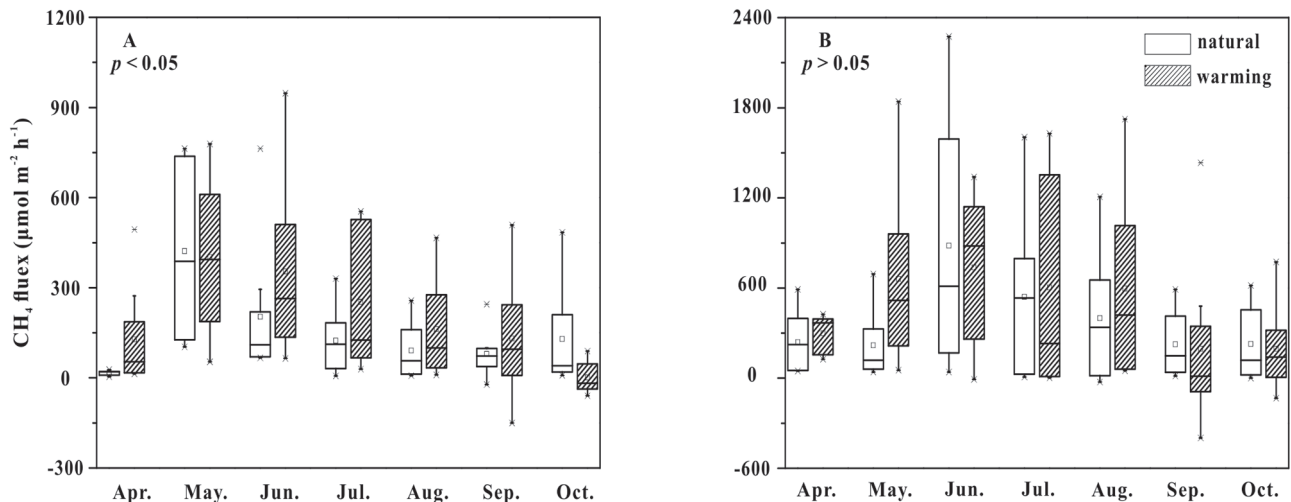


Fig. 1. Methane flux of *Phragmites australis* (A) and *Spartina alterniflora* (B) communities at natural and warming conditions.
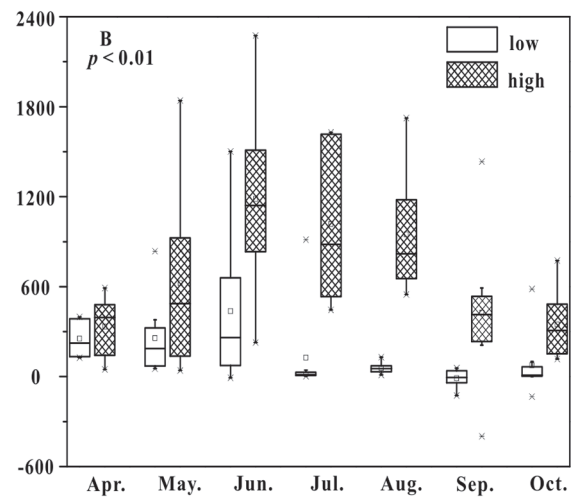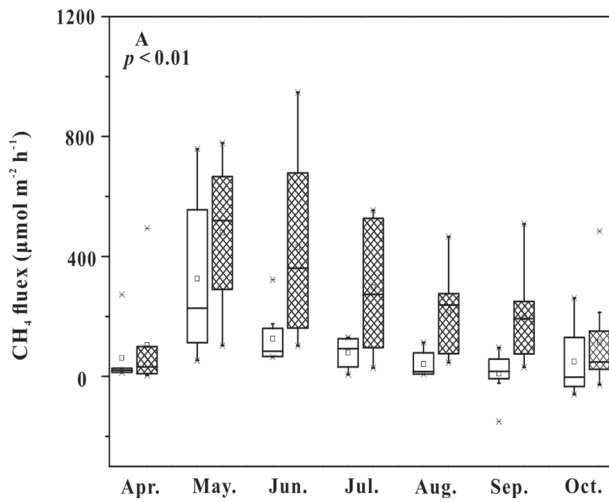
Fig. 2. Methane flux of *Phragmites australis* (A) and *Spartina alterniflora* (B) communities at low-water-level and high-water-level conditions

### Effect of Salinity

The responses of the two communities to differences in salinity were different. Differences in the *P. australis* community were significant ($p < 0.01$). A clear order (5‰ > 15‰ > 0‰ > 30‰) in $CH_4$ flux emerged in the *P. australis* community. By contrast, differences in the *S. alterniflora* community were not significant ($p > 0.05$) (Fig. 3). However, under the polyhaline (30‰) conditions, the average $CH_4$ flux in both communities (79.9 ± 12.5 µmol m$^{-2}$ h$^{-1}$ for *P. australis*; 152.7 ± 33.9 µmol m$^{-2}$ h$^{-1}$ for *S. alterniflora*) was significantly inhibited compared with other environments ($p < 0.01$). Furthermore, at high water levels, average inhibition in a polyhaline (30‰) environment was more significant (52.8 ± 22.9 µmol m$^{-2}$ h$^{-1}$ for *P. australis*; 123.6 ± 38.6 µmol m$^{-2}$ h$^{-1}$ for *S. alterniflora*; $p < 0.01$).

### Interactions between Estuarine Environmental Factors

Both communities showed interactions between the effects of estuarine environmental factors on $CH_4$ flux (Fig. 4). At high water levels, the area of high $CH_4$ flux values (>450 µmol m$^{-2}$ h$^{-1}$) increased (Fig. 4A, C). For the *P. australis* community,

high $CH_4$ flux values (>450 µmol m$^{-2}$ h$^{-1}$) were observed under oligohaline (0–5‰) and mesohaline (5–15‰) conditions at temperatures of 22 to 35°C. As salinity increased, $CH_4$ flux values (>450 µmol m$^{-2}$ h$^{-1}$) gradually decreased, eventually forming areas of low $CH_4$ flux values (<150 µmol m$^{-2}$ h$^{-1}$) at polyhaline (25–30‰) conditions (Fig. 4A). Meanwhile, high $CH_4$ flux values (>1000 µmol m$^{-2}$ h$^{-1}$) at high water levels in the *S. alterniflora* community mainly occurred under oligohaline (0–5‰) conditions at temperatures of 30 to 40°C (Fig. 4C), and low $CH_4$ flux values (<200 µmol m$^{-2}$ h$^{-1}$) were observed under polyhaline (30‰) conditions at temperatures of 18 to 21°C. By contrast, at low water levels, salinity had a more pronounced effect on $CH_4$ flux, resulting in obvious reduction in high $CH_4$ flux values and a significant expansion of low $CH_4$ flux values (Fig. 4B, D). For the *P. australis* community, only under mesohaline (10–20%) conditions at 25°C was the $CH_4$ flux >150 µmol m$^{-2}$ h$^{-1}$ (Fig. 4B). For the *S. alterniflora* community, high $CH_4$ flux values (>400 µmol m$^{-2}$ h$^{-1}$) were mainly observed under mesohaline (10–20‰) at 18 to 26°C, whereas low $CH_4$ flux values (<200 µmol m$^{-2}$ h$^{-1}$) were widely distributed in other conditions (Fig. 4D).
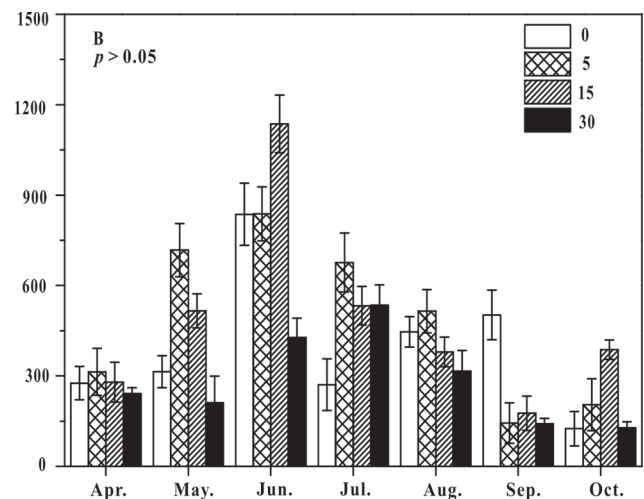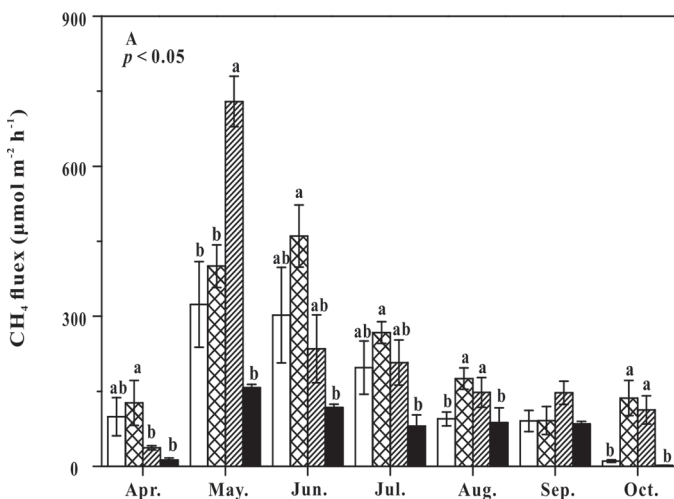


Fig. 3. Methane flux of *Phragmites australis* (A) and *Spartina alterniflora* (B) communities at different salinity conditions. Error bars denote standard error. Lowercase letters denote significant differences based on a one-way ANOVA and Tukey's HSD.
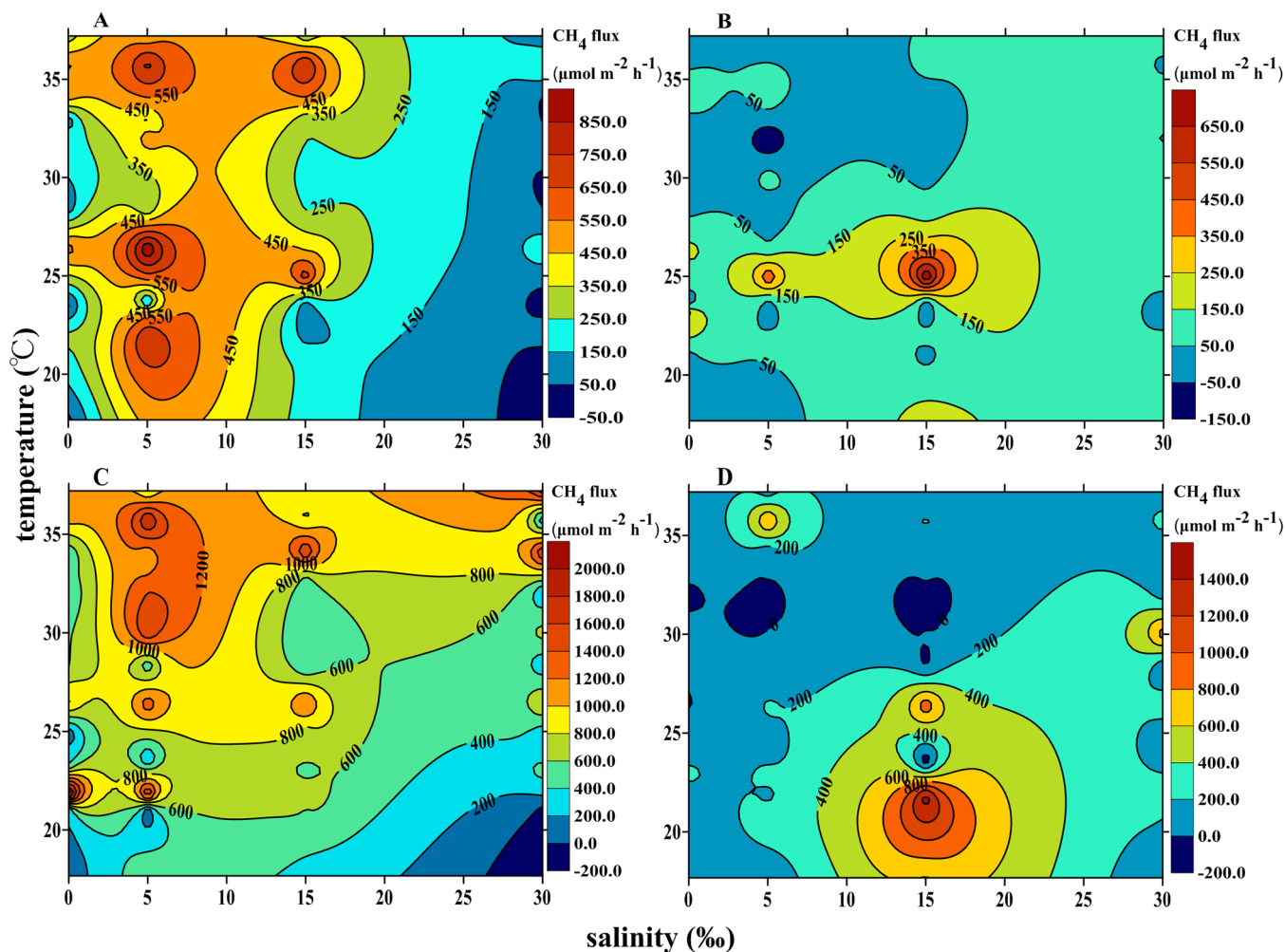
Fig. 4. Interactions between the effects of global change factors on $CH_4$ emissions. (A) Methane flux of *Phragmites australis* at high water levels. (B) Methane flux of *P. australis* at low water levels (C) Methane flux of *Spartina alterniflora* at high water levels. (D) Methane flux of *S. alterniflora* at low water levels.

## DISCUSSION
### Environmental Factors Influence $CH_4$ Flux in Different Vegetation Communities

The activity of methanogens is directly affected by temperature; as temperature increases, methanogens tend to be more active (Jerman et al., 2009). In this study, the $CH_4$ flux in the *P. australis* community showed a positive response to warming ($p < 0.05$), especially during the early and middle stages of the growing season. This indicates that changes in biomass in vegetation during different growth stages can affect $CH_4$ flux in the community. For the same vegetation type, $CH_4$ flux was closely related to vegetation biomass (Duan et al., 2009). Compared with *P. australis*, the biomass of *S. alterniflora* was higher, which led to rapid and organic C accumulation and increased $CH_4$ emissions (Table 1; Fig. 1). However, the effect of warming on $CH_4$ flux in the *S. alterniflora* community was not significant. The higher net primary productivity of *S. alterniflora* is higher than *P. australis*, but the decomposition rate of litter is lower, so the *S. alterniflora* community has a high organic C reserve. Warming had a limited effect on the organic C of the *S. alterniflora* community; there was no significant difference in the organic C content of *S. alterniflora* community in the natural and warming environment ($p > 0.05$) (Table 1), and

the effect on $CH_4$ flux was also not significant ($p > 0.05$) (Fig. 1), indicating that different vegetation types have different responses when the temperature conditions change (Zhang et al., 2012).

As the water level increased, both communities displayed a significant increase in $CH_4$ flux ($p < 0.01$). Methane is produced under strict anaerobic conditions in sediments, and the water level can directly affect gas diffusion rates, microbial activities, and oxygen utilization, which in turn can affect $CH_4$ flux (Chimner, 2004). It has been found that a 15.0-cm reduction in water level in freshwater wetland estuaries can result in a 25% increase in $CH_4$ oxidation in soil and in a consequent decrease in $CH_4$ flux (Bellisario et al., 1999). In the present work, the overlying water level was only 3.0 cm above the soil surface, but the promotional effect on $CH_4$ flux was significant ($p < 0.01$). Due to the formation of a large amount of $CH_4$ in the ground, the $CH_4$ concentration gradient in the underground is established, and $CH_4$ diffuses upward from deeper sediments. Another possible explanation is that an increased water level may develop to increase the vertical accretion of the saline wetlands, increasing the tidal immersion depth and ultimately providing additional inorganic deposits to increase plant productivity (Morris et al., 2002). The increase in vegetation productivity will bring more
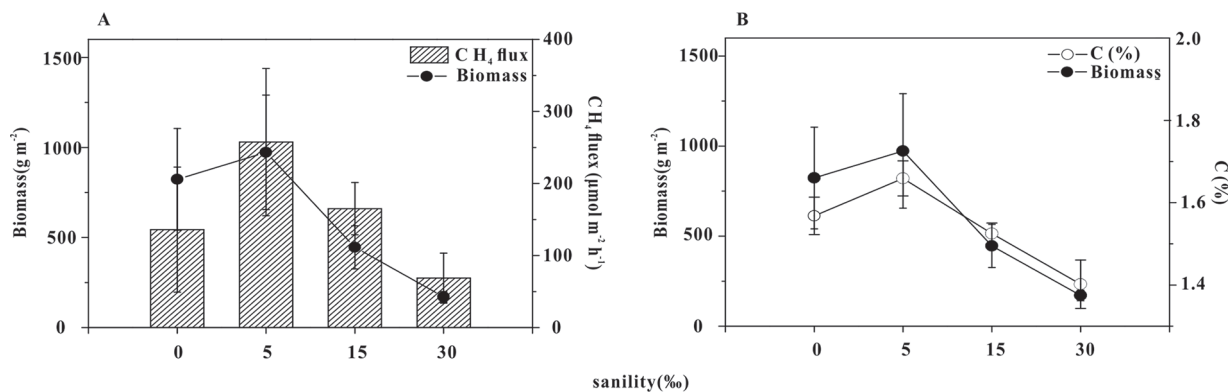
Fig. 5. Methane emissions and biomass (A) and C% and biomass (B) in the *Phragmites australis* community.

organic C sources to methanogens (Stanley and Ward 2010), which will promote the production of $CH_4$.

Compared with $CH_4$ emissions from the freshwater environment (0‰), $CH_4$ emissions from the two communities did not reduce due to the increase of salinity under oligohaline (5‰) conditions (Fig. 3). Studies have shown that the production of $CH_4$ is controlled by low-molecular-weight soluble organic C sources such as acetate, which are easily adsorbed by soil (Liu et al., 2012). The availability of soluble organic C is increased at appropriate salt input (0–10‰) levels in soil. Appropriate salt input can enhance organic C mineralization rate in the soil (Craft et al., 2009) and increase $CH_4$ production (Weston et al., 2011). However, an excessive increase in electron acceptors due to salt input can compete with methanogens for substrates, reducing substrate availability and causing stress to the microbial community (Chidthaisong and Conrad, 2000). In this study, $CH_4$ in the polyhaline (30‰) conditions was significantly inhibited in both vegetation communities ($p < 0.01$). The changes in biomass and $CH_4$ flux in *P. australis* vegetation were similar as salinity increased. When the salinity was 0 to 5‰, biomass and $CH_4$ flux increased, peaking in the oligohaline (5‰) environment, but declined in polyhaline (15–30‰) conditions (Fig. 5A). Salinity can negatively affect plant productivity and biomass by the accumulation of $HS^-$ (Koch, 1990) and the inhibition of nutrient uptake (Bradley and Morris, 1991). In most locations, the change in organic C substrate in tidal wetlands follows the change in plant productivity and biomass (Wie ski et al., 2010). The decomposition of wetland plants provides the ecosystem a major source of organic C, a universal electron donor driving many heterotrophic microbial processes (Stanley and Ward 2010). In our study, we also found that organic C changes with biomass in different salinity environments (Fig. 5B). This indicates that $CH_4$ flux is affected by salinity through effects on the biomass amount of vegetation. Previous studies showed that 10‰ salinity is the threshold for effective geochemical cycling in wetland soil. In an environment with <10‰ salinity (Weston et al., 2006), the manner of organic matter mineralization can rapidly transform from the methanogenesis to iron reduction and sulfate reduction (Weston et al., 2006), and other studies showed that salinity >13‰ can affect $CH_4$ emissions (Bartlett et al., 1987). In the present study, the $CH_4$ flux in the *S. alter-*

*niflora* community at a salinity of 15‰ was higher than that in a freshwater environment (Fig. 3B). There are many factors affecting soil microbial communities in wetland ecosystems, and establishing the threshold of the effects of salinity requires more in-depth research.

Both vegetation communities were a source of atmospheric $CH_4$, except at the end of the growing season. There are differences in $CH_4$ emission fluxes for different types of wetlands in different regions. The $CH_4$ emissions flux in wetlands is the combined effect of various key environmental factors including temperature, water level, salinity, and organic C content. The $CH_4$ emissions from both communities were moderate in this study compared with tidal wetlands worldwide (Table 2).

## Interactions between Factors in the Context of Estuarine Environmental Factors

In this study, water level was found to be an important factor affecting $CH_4$ flux in different vegetation communities. The $CH_4$ flux of the two communities at high water level was significantly higher than that at low water level ($p < 0.01$) (Fig. 2 and 4). In addition to increasing $CH_4$ flux in vegetation communities, higher water levels can alter the intensity and sensitivity of $CH_4$ in response to changes in temperature and salinity. In a previous study on the interactions between the effects of global change factors on estuarine wetland ecology, changes in wetland hydrological characteristics caused by sea level rise were considered the dominant factor affecting the balance between ecological function and material circulation (Cao et al., 1996; Casanova and Brock, 2000). The $CH_4$ flux from wetlands is affected by interactions between temperature and water level. When the soil is saturated with water, $CH_4$ flux changes with temperature (Roulet et al., 1992), indicating that the effect of temperature on $CH_4$ flux is more pronounced under high water levels, resulting in higher $CH_4$ emissions. By contrast, at low water levels, the effect of warming on $CH_4$ flux is reduced, and the effect of salinity is more obvious, resulting in lower $CH_4$ flux values.

## Implications for the Tidal Wetlands

Tidal wetlands cover a total area of ~538,000 to 552,000 $km^2$ (Mcleod et al., 2011) but are considered important sinks of C in the biosphere (Nahlik and Fennessy, 2016). Although tidal wetlands

**Table 2. Comparison of methane emissions in this study and from tidal wetlands worldwide**

| Study area | Vegetation type | | Source |
|---|---|---|---|
| | *Phragmites australis* | *Spartina alterniflora* | |
| | ——— μmol m$^{-2}$ h$^{-1}$ ——— | | |
| Bay Tree Creek Salt Marsh, USA | 7.08† | | Bartlett et al. (1985) |
| Scheldt estuary, Belgium | | 158.0† | van der Nat and Middelburg (1998) |
| Scheldt estuary, The Netherlands | | 537.0† | van der Nat and Middelburg (2000) |
| Jiuduansha salt marsh, Yangtze River estuary, China | 10.0–70.0† | 13.1–41.3† | Cheng et al. (2007) |
| Min River estuary, China | 688.0† | 271.0† | Tong et al. (2010) |
| Liaohe Delta, China | | 32.5† | Huang et al. (2001) |
| Yangtze River estuary, China | | 82.6–728.0† | Ma et al. (2012) |
| Minjiang estuary, China | | 52.5–400.0† | Wang et al. (2015) |
| Nebraska Reed marshes, USA | | 570.0† | Kim et al. (2010) |
| Bay Tree Creek Salt Marsh, USA | 159.0† | | Bartlett et al. (1987) |
| Wanggang estuary, China | 172.0† | | Ding et al. (2010) |
| Wanggang estuary, China | 46.9–229.4† | | Zhang and Ding (2011) |
| Bay of Fundy, New Brunswick, Canada | 1.48† | 1.56† | Magenheimer et al. (1996) |
| Yangtze River estuary, China | 40.0–851.0† | 12.5–123.0† | Zeleke et al. (2013) |
| Min River estuary China | 1768.0–3565.0† | 1140.0–3500.0† | Tong et al. (2012) |
| Gulf of Bothnia, Baltic sea,Finland | | 36.5–65.1† | Silvennoinen et al. (2008) |
| Narragansett Bay, Rhode Island, USA | | 15.0–1254.0 | Martin and Moseman-Valtierra (2015) |
| Min River estuary, China | | −963.0 to 1281.0† | Yang et al. (2017) |
| Liaohe Delta, China | | 43.8–156.3† | Olsson et al. (2015) |
| Yangtze River estuary, China | 141.0 ± 21.5 | 502.0 ± 65.3 | this study |

† Indicates unit conversion.

act as large C sinks, they are also important sources of greenhouse gases, such as $CH_4$ (Bloom et al., 2017). As a result, tidal wetlands play an important role in regulating the C cycle at a global scale and quantifying the C budgets of wetlands, including C stores, uptake, and emission (Lu et al., 2017). Based on the findings of this study, increases in the temperature, water level, and oligohaline (5‰) conditions may have a greater impact on the methanogenesis from different vegetation communities. In the present study, the average $CH_4$ flux in *P. australis* and *S. alterniflora* communities during warming and high-water-level conditions were 321.0 ± 49.3 and 810.0 ± 98.6 μmol m$^{-2}$ h$^{-1}$, respectively. The findings of this study indicate that $CH_4$ emissions from tidal wetlands will be further strengthened by the combined effect of increased global warming and sea level rises in the future.

## CONCLUSIONS

The Yangtze has one of the biggest estuaries in the world, overlying extensive tidal flats that are highly vulnerable to changes in estuarine environmental factors. This study represents the first attempt to disentangle the effects of increasing temperature, increasing water level, increasing salinity, and the combination thereof on $CH_4$ emissions using experimental tidal mesocosms. Results indicated that global climate change has significantly changed the $CH_4$ emissions pattern of the estuarine coastal wetlands. In addition, there were differences in the corresponding changes in environmental factors among different vegetation communities. Warming can significantly increase $CH_4$ emissions from the *P. australis* community ($p < 0.05$). At high water levels, $CH_4$ emissions from both communities increased significantly ($p < 0.01$). The biomass amount of the vegetation community was affected by salinity, which leads to higher $CH_4$ emissions at oligohaline (0–5‰) conditions. The interaction between high water level and warming was a significant factor affecting the $CH_4$ emission of the community. At the high water level, the effect of high temperature was more obvious. On the contrary, the inhibition of salinity at the lower water level was more dominant.

In addition to the observed changes in $CH_4$ emissions, this study identified other potentially important biogeochemical responses to simulated estuarine environmental factors. Most notably, $CH_4$ emissions from tidal wetlands will be further strengthened by the combined effect of increased global warming and sea level rises. To fully understand the implications of estuarine environmental factors on the Yangtze estuary, field studies that incorporate C inputs and natural feedback mechanisms are needed.

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