

Diurnal variations of carbon dioxide, methane, and nitrous oxide fluxes from invasive *Spartina alterniflora* dominated coastal wetland in northern Jiangsu Province

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Abstract

The invasions of the alien species such as *Spartina alterniflora* along the northern Jiangsu coastlines have posed a threat to biodiversity and the ecosystem function. Yet, limited attention has been given to their potential influence on greenhouse gas (GHG) emissions, including the diurnal variations of GHG fluxes that are fundamental in estimating the carbon and nitrogen budget. In this study, we examined the diurnal variation in fluxes of carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) from a *S. alterniflora* intertidal flat in June, October, and December of 2013 and April of 2014 representing the summer, autumn, winter, and spring seasons, respectively. We found that the average CH₄ fluxes on the diurnal scale were positive during the growing season while negative otherwise. The tidal flat of *S. alterniflora* acted as a source of CH₄ in summer (June) and a combination of source and sink in other seasons. We observed higher diurnal variations in the CO₂ and N₂O fluxes during the growing season (1 536.5 mg CO₂ m⁻² h⁻¹ and 25.6 μg N₂O m⁻² h⁻¹) compared with those measured in the non-growing season (379.1 mg CO₂ m⁻² h⁻¹ and 16.5 μg N₂O m⁻² h⁻¹). The mean fluxes of CH₄ were higher at night than that in the daytime during all the seasons but October. The diurnal variation in the fluxes of CO₂ in June and N₂O in December fluctuated more than that in October and April. However, two peak curves in October and April were observed for the diurnal changes in CO₂ and N₂O fluxes (prominent peaks were found in the morning of October and in the afternoon of April, respectively). The highest diurnal variation in the N₂O fluxes took place at 15:00 (86.4 μg N₂O m⁻² h⁻¹) in June with an unimodal distribution. Water logging in October increased the emission of CO₂ (especially at nighttime), yet decreased N₂O and CH₄ emissions to a different degree on the daily scale because of the restrained diffusion rates of the gases. The seasonal and diurnal variations of CH₄ and CO₂ fluxes did not correlate to the air and soil temperatures, whereas the seasonal and diurnal variation of the fluxes of N₂O in June exhibited a significant correlation with air temperature. When N₂O and CH₄ fluxes were converted to CO₂-e equivalents, the emissions of N₂O had a remarkable potential to impact the global warming. The mean daily flux (MF) and total daily flux (TDF) were higher in the growing season, nevertheless, the MF and TDF of CO₂ were higher in October and those of CH₄ and N₂O were higher in June. In spite of the difference in the optimal sampling times throughout the observation period, our results obtained have implications for sampling and scaling strategies in estimating the GHG fluxes in coastal saline wetlands.

Key words: methane, nitrous oxide, carbon dioxide, diurnal variation, *S. alterniflora*

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1 Introduction

Carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) are active greenhouse gas (GHG) that contributes tremendously to global warming (IPCC, 2013). The measurement of GHG fluxes in various ecosystems is fundamental to estimate their effects on global warming (IPCC, 2013). Considerable efforts have been made to investigate GHG fluxes in various coastal wetlands, such as river estuary (Sun et al., 2013; Cheng et al., 2010), coastal lagoon (Hirota et al., 2007), mangrove swamp (Chen et al., 2010; Allen et al., 2011), and tidal freshwater marsh (Van der Nat and Middelburg, 2000). However, in most of these previous studies, field samplings were conducted in the daytime,

e.g., from 8:00 to 10:00 and from 6:00 to 18:00, in order to minimize the effects of diurnal variations of GHG emissions (Allen et al., 2007; Cheng et al., 2010; Yu et al., 2012; Zhang et al., 2015). Yet, the flux rates do not stay the same throughout the whole day. Consequently, their estimates of the total emissions were unlikely to be unbiased as they missed the indispensable flux rates at the other times of a day, especially when they conducted the sampling at only one part of the diurnal cycle (Morin et al., 2014; Maljanen et al., 2002). Therefore, it is urgent to quantify the GHG fluxes on a diurnal scale to evaluate accurately the seasonal and annual variations of the GHG emissions and the budget of carbon and nitrogen.

There are numerous studies about the diurnal variation in the

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fluxes of CH₄ (Nakano et al., 2000; Duan et al., 2005; Ding and Cai, 2007; Chen et al., 2010; Zhang et al., 2011), N₂O (Zhu et al., 2008; Yu et al., 2012), and CO₂ (Parkin and Kaspar, 2003). Mikkilä et al. (1995) reported a clear diurnal variation of N₂O fluxes: the peaks occurred in the afternoon and N₂O emissions in the daytime were as much as five times higher than those over the night. They also found that the ratio of average daytime CH₄ fluxes to nighttime CH₄ fluxes varying with the plant community from 2 to 20 (Mikkilä et al., 1995). With the same pattern as N₂O emissions, the highest diurnal variation in the fluxes of CO₂ and CH₄ took place in the mid-afternoon or around noon time (Parkin and Kaspar, 2003; Morin et al., 2014; Zhang et al., 2015) and minimum CO₂ emission occurred in the night or in the early morning (Maljanen et al., 2002; Parkin and Kaspar, 2003; Zhang et al., 2015). Despite that there are a number of studies focused on the three kinds of GHG fluxes together on neap and spring tide days in estuarine marsh (Tong et al., 2013) and in cultivated and forested organic boreal soils (Maljanen et al., 2002), studies are scarce on diurnal variations in the three GHG fluxes together in coastal saline wetlands.

Many studies have been performed on the impacts of different types of vegetation, land use and coverage, and tide days on the GHG emissions (Maljanen et al., 2002; Zhang and Ding, 2011; Tong et al., 2013; Zhang et al., 2015). For instance, some previous studies have focused on the fluxes in the stages of panicle, flowering, fruiting, and maturing (Wang et al., 2005), at different weather conditions (Mikkilä et al., 1995), and in various tidal flats (Zhang et al., 2015; Sun et al., 2013). Yet, the knowledge about the diurnal variations of the GHG fluxes in various stages of vegetation growth is still limited. Moreover, many previous studies preferred to measure the GHG emissions during the plant growing seasons (Duan et al., 2005; Hirota et al., 2007; Chen et al., 2010; Zhang and Ding, 2011). However, the GHG fluxes in wintertime or non-growing season should be never neglected as they accounted for roughly 36% to 38% of the total annual flux (Morin et al., 2014).

Diurnal variations in GHG fluxes are affected by many environmental factors, including the air and soil temperatures (Nieveen et al., 1998; Maljanen et al., 2002; Parkin and Kaspar, 2003; Hirota et al., 2007; Zhu et al., 2008), water table depth (Hirota et al., 2007; Zhu et al., 2008), solar radiation (Duan et al., 2005; Yu et al., 2012; Mikkilä et al., 1995) and so on. *Spartina alterniflora*, a perennial grass with C₄-photosynthesis, was deliberately introduced to China in 1979 (Qin and Zhong, 1992) and planted across the intertidal zones in Jiangsu since 1982 (Liu et al., 2007) to protect the seawall and accelerate deposition (Cheng et al., 2006; Tong et al., 2012). No conclusion has been made so far about the function of *S. alterniflora* in the regulation of the GHG fluxes, which is important to determine the function of coastal wetlands as sources or sinks of carbon and nitrogen (Zhang et al., 2013). Some studies have investigated the effects of plants on the mechanism of CH₄ emission (Van der Nat and Middelburg, 2000; Duan et al., 2005) and the process of labile carbon to the root zone and activity of stomatal conductance (Morin et al., 2014) caused by the invasion of *S. alterniflora* (Yuan et al., 2014; Cheng et al., 2010). Emery and Fulweiler (2014) and Cheng et al. (2007) compared the fluxes of CH₄ and N₂O from *S. alterniflora* and *Phragmites* flats. Chen et al. (2012) showed that CO₂ emissions increased with the invasion of *S. alterniflora* and that the CO₂ emissions of *S. alterniflora* are higher than that of *Phragmites* flats because of high soil microbial respiration. There are few studies investigating the three GHG emissions in *S. alterniflora*-covered flats together. We chose *S. alterniflora* for the

measurement of diurnal fluxes of CO₂, CH₄, and N₂O across the different growth stages also because of its widespread distribution in the southeast coastal wetland in China.

Diurnal variations in the GHG fluxes in different seasons should be considered in assessing annual GHG fluxes. To estimate accurately the balance of CH₄, N₂O, and CO₂ for different stages of *S. alterniflora* growth, the short-term changes in the fluxes have to be determined. The objectives of this study are: (1) to determine the diurnal variations of CO₂, CH₄, and N₂O fluxes in different stages of *S. alterniflora* growth; (2) to assess the effects of plant traits and environmental variables (air temperature, soil temperature, and aboveground biomass) on diurnal variations of the GHG emissions; and (3) to determine the optimal field sampling time that would minimize the effect of diurnal variations on the GHG emissions.

2 Materials and methods

2.1 The study site

Mainly covered with *S. alterniflora*, the study area is located in the intertidal flat in the core region of Yancheng National Nature Reserve for Wetland and Rare birds (32°48'47"N–34°29'28"N; 119°53'45"E–121°18'12"E) at Sheyang, Jiangsu Province, Southeast China. The annual precipitation is 1 000 mm (about 40% to 50% precipitation fall between June and August), the annual mean temperature is 14°C and the seasonal mean temperatures are 13.6°C, 28.9°C, 14.6°C, and –2.0°C for spring, summer, autumn, and winter, respectively (Xu et al., 2014). The vegetation includes *S. alterniflora*, *Suaeda glauca*, *Aeluropus litoralis*, *Imperata cylindrica*, and *Phragmites australis*. They are developed from the sea to the inland and are rarely disturbed by human activities. The coastal marshes are typical alluvial mudflats with semidiurnal tidal periodicity (Yuan et al., 2014). The local soil is composed of 19.6% sand, 40.1% silt, and 40.3% clay (Liu et al., 2007). The reserve is not only the first and largest coastal wetland reserve and a typical coastal salt marsh ecosystem in China but also a main habitat of other wild animals (e.g., *Hydropotes inermis* and red-crowned crane) (Zhou et al., 2003).

2.2 Sample collection and analysis

The fluxes of CO₂, CH₄, and N₂O were measured using a static opaque chamber and modified gas chromatograph (Agilent 7890) (Livingston and Hutchinson, 1995). Three replicate plots, which were set up in *S. alterniflora*-covered flats distributed from the sea to the inland, were simultaneously observed for each field measurement. The distance between each plot was approximately 100 m. The average height of *S. alterniflora* was more than 1.5 m. Chambers were built with a bottom collar covering an area of 50 cm×50 cm and a depth of 30 cm. Three PVC chambers (50 cm×50 cm×50 cm) were added, with the connected chamber open on the bottom and the top and the upper chamber open on the bottom only (Xu et al., 2014). During the observation time, the bottom collar was inserted permanently into the marsh sediment. The upper chamber was equipped with an electric fan to ensure complete mixing of the internal air and to reduce changes in internal temperature. A mercury thermometer was used to measure the internal temperature of the chamber. To prevent the increase of air temperature inside the chamber, insulated cotton quilts were utilized. Site selection and chamber placement minimized the differences in microclimate and represented the characteristics of *S. alterniflora*.

Liao et al. (2007) stated that the growing season for *S. alterniflora* is 270 d, which increases with the time of invasion. In this

study, we conducted the diurnal measurements in four periods: June 16–17, 2013, October 19–20, 2013, December 23–24, 2013 and April 1–2, 2014. June and October represent the growing season and December and April represent the non-growing season of *S. alterniflora*. Gas samples were collected every 3 h from 9:00 of the first day to 6:00 of the subsequent day in Beijing standard time (GMT+8) (Chen et al., 2010). Each measurement consisted of eight sampling campaigns. Gas samples were obtained with 100 mL polypropylene syringes that are equipped with three-way stopcocks at 0, 10, 20, and 30 min intervals. The samples were stored in the gas-sampling bag, and analyzed within 3 days. Except for October, when an average of 5 cm to 10 cm layer of water was observed above the sediment surface, however, for the other sampling dates the sediment surface was not covered with water.

The mixing ratios of CH₄ and N₂O were simultaneously analyzed using a modified Agilent 7890 equipped with a flame ionization and electron capture detectors. The configuration of gas chromatograph and procedures for simultaneous measurement of CH₄ and N₂O fluxes were described in detail in the previous studies (Liu et al., 2014).

We also measured the growth parameters of *S. alterniflora*, including the plant height and plant biomass. Soil moisture was not included as a measure in this study because no significant difference was found over the relatively short-time (diurnal) scale. Only the data on soil and air temperatures, which varied in the diurnal scale, were used to test the relationships between the GHG and environmental variables. The soil temperature in June was not measured in our study.

2.3 Conversion to CO₂-equivalents

Global warming potential (*GWP*) is a measure of how much a given mass of GHG is needed to contribute to a certain global warming. Gaseous emissions were converted to CO₂-equivalents by using *GWP*, an index defined as the cumulative radiative force between the present and a chosen later time horizon caused by a unit mass of gas that is currently emitted. It is used to compare the effectiveness of each GHG in trapping heat in the atmosphere (IPCC, 2013). Based on the data over a 100-year period, the *GWP* coefficients for CH₄ and N₂O are 28 and 265, respectively, when the *GWP* value for CO₂ is considered as 1 (IPCC, 2013).

$$GWP = x + 28y + 265z, \quad (1)$$

where *x* is the CO₂ fluxes (mg CO₂ m⁻² h⁻¹), *y* is the CH₄ fluxes (mg CH₄ m⁻² h⁻¹), and *z* is the N₂O fluxes (μg N₂O m⁻² h⁻¹). *GWP* means the global warming potential (mg CO₂-equivalent m⁻² h⁻¹).

2.4 Data treatment and statistics

To evaluate the difference of data at different time of the day, one-way analysis of variation (ANOVA) was employed. Differences were considered significant if *p*<0.05. Pearson correlation was performed to analyze the correlations between GHG fluxes and environmental parameters and plant biomass. To scientifically and consistently compare daytime and nighttime average emissions, the average daytime emissions were calculated using fluxes collected from 7:00 to 19:00. The basis for the day/night division was the same throughout the observation period.

$$MDF \text{ (mean daytime flux)} = (MF(9:00) + MF(12:00) + MF(15:00) + MF(18:00)) / 4, \quad (2)$$

$$MNF \text{ (mean nighttime flux)} = (MF(21:00) + MF(24:00) + MF(3:00) + MF(6:00)) / 4. \quad (3)$$

Total daily flux (*TDF*) was calculated using the following formula:

$$TDF = \left(\sum_{i=1}^{n=8} F_i \right) \times 3, \quad (4)$$

where *i* is the measuring time and *F_i* means the flux at different times of the day.

To minimize deviation caused by diurnal variation, the optimal time to measure GHG fluxes was needed to select. There were two steps to determine the optimal time. First, the *F_i* measured in different times of day were compared, and the time with the closest mean flux values was chosen. Second, the total flux deviation (*TFD*) was calculated by summing up the flux deviations in CH₄, N₂O, and CO₂ as follows:

$$TFD = \sum_{i=1}^{n=8} \sqrt{\left(\frac{MF_{CH_4} - F_{i-CH_4}}{MF_{CH_4}} \right)^2} + \sqrt{\left(\frac{MF_{N_2O} - F_{i-N_2O}}{MF_{N_2O}} \right)^2} + \sqrt{\left(\frac{MF_{CO_2} - F_{i-CO_2}}{MF_{CO_2}} \right)^2}, \quad (5)$$

where *MF* is the mean flux, and *F_i* is the flux at different times of the day.

3 Results and discussion

3.1 Environmental factors and vegetation characteristics

The diurnal patterns of air and soil temperatures exhibited unique peaks in different seasons (Fig. 1). In June and October, the maximum air temperatures were observed at 9:00 and remained about the same until 15:00. However, the average air temperatures in December and April peaked later (at 12:00) than the growing season. The lowest air and soil temperatures were both observed in the early morning (3:00 and 6:00, depending on the season). Diurnal changes in soil temperature were lower than those in air temperature (e.g., the variation coefficients (CV) for air and soil temperatures were 31.1% and 12.9%, respectively, in October and 34.9% and 15.8% in April) (Table 1). However, the changes in soil temperature at 5 cm depth closely paralleled to the changes in air temperature. Significant correlations (October: *r*=0.909, *p*<0.01, *n*=8; December: *r*=0.729, *p*<0.05, *n*=8) were found between them. Soil temperature was higher than air temperature during nighttime (18:00 to 6:00), whereas an opposite result was found in the daytime across the study period. A significant correlation (*r*=0.912, *p*<0.001, *n*=24) likewise existed across the observation.

In Table 2, seasonal vegetation characteristics were recorded. The plant height and plant biomass increased with the growth of *S. alterniflora* and then gradually decreased upon entering into the non-growing season.

3.2 Diurnal variations of CH₄ fluxes

Diurnal variations of CH₄, N₂O, and CO₂ fluxes measured at various seasons showed no stable pattern (Fig. 2). As mentioned above, June and October were regarded as the growing season of *S. alterniflora* and December and April the non-growing season. In June, the maximum CH₄ emission was 0.506 mg CH₄ m⁻² h⁻¹ at 21:00, and the minimum emission rate appeared at 15:00 (Fig. 2a). The *MDF* and *MNF* were (0.148±0.088) and (0.251±0.180) mg

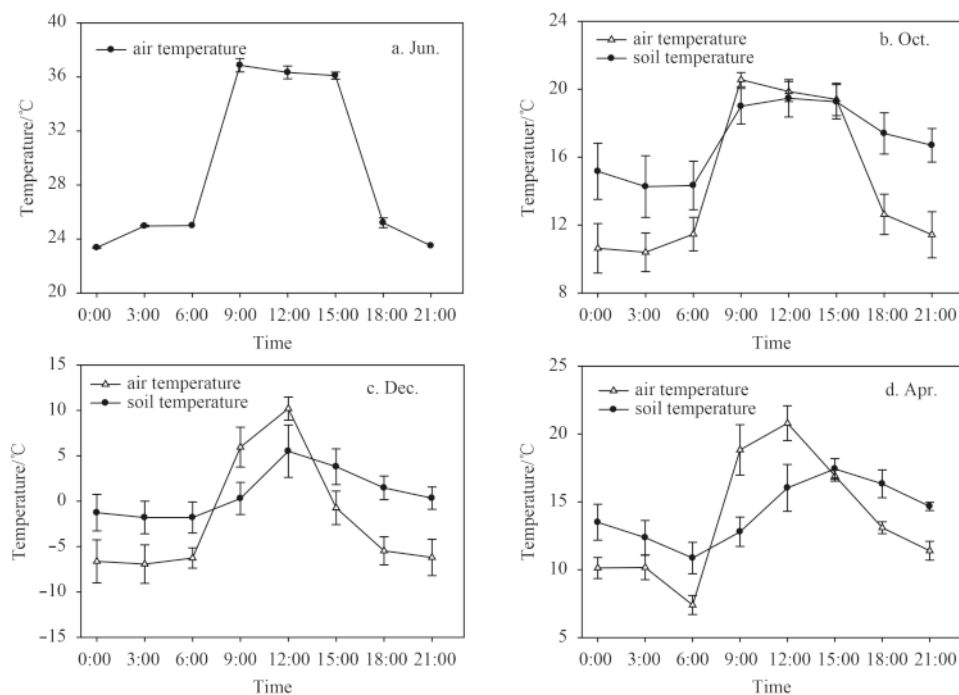


Fig. 1. Diurnal variations of air temperature and soil temperature (5 cm) in *S. alterniflora*-covered flats during different seasons in Yancheng coastal saline wetland. Bars represent the mean±stand error ($n=3$). Positive values represent emission and negative values represent uptake. a. June (summer) 2013, b. October (autumn) 2013, c. December (winter) 2013, and d. April (spring) 2014. Soil temperature was missed in June 2013.

Table 1. Diurnal variation coefficient (CV) in different seasons

CV	CO ₂	CH ₄	N ₂ O	Air temperature	Soil temperature
Apr.	0.72	N	0.62	0.35	0.16
Jun.	0.47	0.71	1.05	0.22	N
Oct.	1.14	8.67	0.56	0.31	0.13
Dec.	0.28	N	0.50	N	3.27

Note: The average CH₄ in April and December, and average air temperature in December was below the zero, the CV cannot be calculated from the negative values. The soil temperature in June was not measured (N).

Table 2. Seasonal changes in the characteristics of *S. alterniflora* (mean±standard error)

Sampling time	Plant height/cm	Plant biomass/kg·m ⁻²
Apr.	99±16	3.0±0.6
Jun.	79.2±11.7	2.3±1.1
Oct.	170±21	5.5±2.4
Dec.	121±27	3.7±0.8

CH₄ m⁻² h⁻¹, respectively (Table 3). In October, the CH₄ flux ranged from -1.011 to 0.448 mg CH₄ m⁻² h⁻¹, with an obvious uptake at 6:00 (-1.011 mg CH₄ m⁻² h⁻¹) (Fig. 2b). The average CH₄ flux was 0.146 mg CH₄ m⁻² h⁻¹ in the daytime and -0.102 mg CH₄ m⁻² h⁻¹ during the night (Table 3). The average diurnal CH₄ fluxes were 0.199 and 0.022 mg CH₄ m⁻² h⁻¹ in June and October, respectively (Table 4). Significant differences ($p<0.001$) for CH₄ emissions were observed between different times of the day during the growing season. In December, an obvious uptake of CH₄ occurred at 9:00, with the value of -0.327 mg CH₄ m⁻² h⁻¹. The highest emission was observed at 21:00 (0.205 mg CH₄ m⁻² h⁻¹) (Fig. 2c). The *S. alterniflora*-covered flats shifted from a sink of CH₄ fluxes (-0.076 mg CH₄ m⁻² h⁻¹) in the daytime to a source of CH₄ (0.063 mg CH₄ m⁻² h⁻¹) (Fig. 2c, Table 3). In April, CH₄ flux was found nearly stable except for an obvious uptake (-0.469 mg CH₄ m⁻² h⁻¹) at 15:00, the peak emission was found at 3:00 during

the night (Fig. 2d).

According to our field observations, an average of 5 cm water logging above the topsoil was found during the investment in October. The average diurnal CH₄ fluxes were higher during the nighttime than in the daytime in all the time but October (Table 3, Fig. 2). Mikkilä et al. (1995) found that nighttime CH₄ emission was twice as high as the daytime rates in the dry plant communities owing to a delay in the supply of root-exuded substrate for anaerobic bacteria. Additionally, a weak consumption was observed during the night in October (Table 3). However, this result was not consistent with the findings of previous studies (Chanton et al., 1997; Duan et al., 2005; Ding and Cai, 2007; Morin et al., 2014), indicating that CH₄ fluxes exhibited a clear diurnal variation. That is, the flux increased early in the morning, reached a unique peak at 9:00 or at noontime, and then declined in the afternoon. Overall, in our study, CH₄ was emitted during the growing season (June: 0.199 mg CH₄ m⁻² h⁻¹; October: 0.022 mg CH₄ m⁻² h⁻¹) of *S. alterniflora*. By contrast, CH₄ was absorbed in the non-growing season (December: -0.007 mg CH₄ m⁻² h⁻¹; April: -0.057 mg CH₄ m⁻² h⁻¹) (Table 4) as a result of the daytime CH₄ consumption (Table 3, Fig. 2). The high source of substrate for methanogenesis (Table 2) and the well-developed gas transport during the growing season were the causes (Joabsson et al., 1999; Allen et al., 2011). Nevertheless, Morin et al. (2014) argued

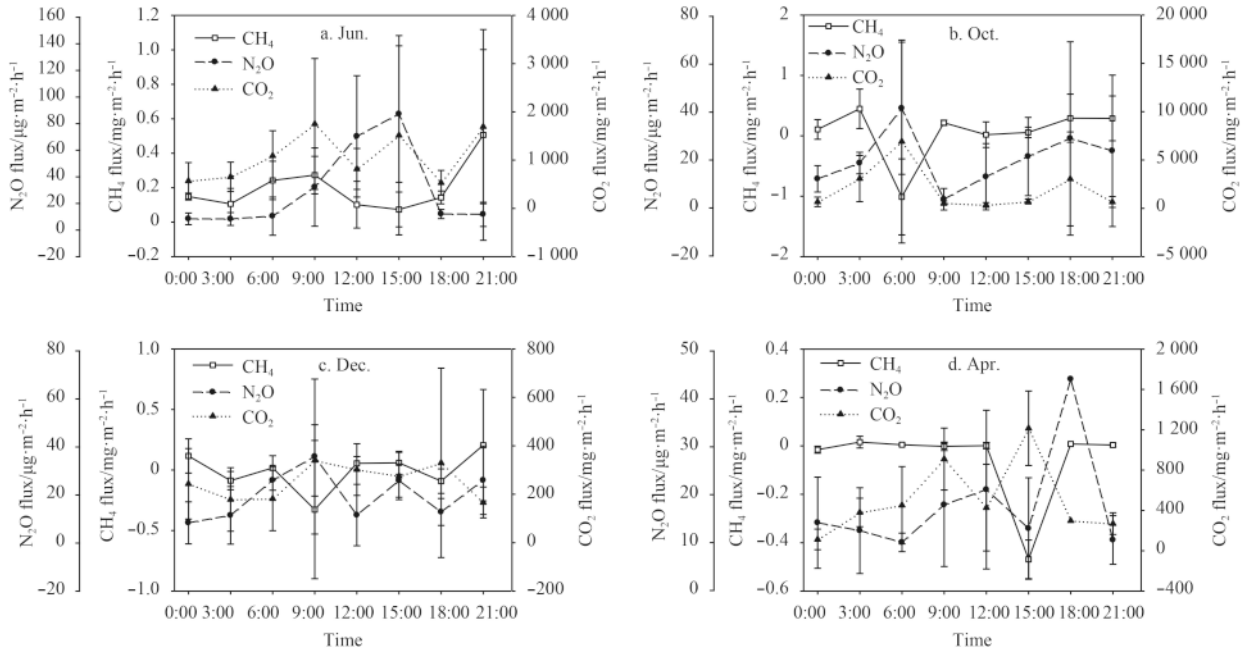


Fig. 2. Diurnal variations of CH₄, N₂O, and CO₂ in *S. alterniflora*-covered flats during different seasons in Yancheng coastal saline wetland. Bars represent the mean±stand error (n=3). Positive values represent emission and negative values represent uptake. a. June (summer) 2013. b. October (autumn) 2013, c. December (winter) 2013, and d. April (spring) 2014.

Table 3. Diurnal variations of three GHG fluxes during the daytime and nighttime in March, June, October, and December (mean±standard error)

	Apr.			Jun.			Oct.			Dec.		
	CH ₄ /mg·m ⁻² ·h ⁻¹	N ₂ O/μg·m ⁻² ·h ⁻¹	CO ₂ /mg·m ⁻² ·h ⁻¹	CH ₄ /mg·m ⁻² ·h ⁻¹	N ₂ O/μg·m ⁻² ·h ⁻¹	CO ₂ /mg·m ⁻² ·h ⁻¹	CH ₄ /mg·m ⁻² ·h ⁻¹	N ₂ O/μg·m ⁻² ·h ⁻¹	CO ₂ /mg·m ⁻² ·h ⁻¹	CH ₄ /mg·m ⁻² ·h ⁻¹	N ₂ O/μg·m ⁻² ·h ⁻¹	CO ₂ /mg·m ⁻² ·h ⁻¹
Day												
Min	-0.469	12.9	298.1	0.074	11.8	530.7	0.019	3.8	354.0	-0.327	11.4	275.2
Max	0.008	43.8	1 214.7	0.273	86.4	1 749.7	0.292	28.9	3 041.7	0.059	35.6	339.8
Mean	-0.115±0.236	23.9±13.7	712.6±425.8	0.148±0.088	50.0±34.1	1 153.5±573.8	0.146±0.130	16.8±10.8	1 159.8±1 262.7	-0.076±0.181	21.3±11.5	311.6±29.0
Night												
Min	-0.016	10.1	112.4	0.106	8.1	570.3	-1.011	12.2	658.2	-0.087	8.2	165.9
Max	0.016	14.1	451.8	0.506	11.6	1 693.7	0.448	41.4	6 912.4	0.205	25.8	242.9
Mean	0.002±0.013	11.8±1.9	303.3±147.8	0.251±0.180	9.5±1.7	1 002.4±514.2	-0.102±0.631	24.0±12.5	2 830.8±2 950.2	0.063±0.126	17.8±9.3	192.3±34.5

Table 4. The total daily flux (TDF), the mean flux (MF) and GWP (calculated by CO₂ equivalents) on a daily scale in different seasons (mean±standard error)

		Apr.	Jun.	Oct.	Dec.
		TDF	CO ₂ /mg·m ⁻² ·d ⁻¹	11 594.7±10 016.6	25 871.1±10 892.5
	CH ₄ /mg·m ⁻² ·d ⁻¹	-1.376±2.191	4.784±2.074	0.618±6.087	-0.156±2.290
	N ₂ O/μg·m ⁻² ·h ⁻¹	340.8±211.8	713.9±197.0	525.2±129.2	426.8±113.8
MF	CO ₂ /mg·m ⁻² ·h ⁻¹	506.3±398.2	1 078.0±453.9	1 995.3±2 282.8	251.9±47.9
	CH ₄ /mg·m ⁻² ·h ⁻¹	-0.057±0.091	0.199±0.086	0.022±0.442	-0.007±0.095
	N ₂ O/μg·m ⁻² ·h ⁻¹	15.1±8.3	29.7±8.2	21.5±3.9	17.8±4.7
GWP	CO ₂ /mg·m ⁻² ·h ⁻¹	506.3±398.2	1 078.0±453.9	1 995.3±2 282.8	251.9±47.9
	CH ₄ /mg·m ⁻² ·h ⁻¹	-1.618±2.546	5.581±2.420	0.616±7.786	-0.182±2.672
	N ₂ O/mg·m ⁻² ·h ⁻¹	4.0±2.2	7.9±2.2	5.7±1.0	4.7±1.3
	Total	508.382	1 091.481	2 001.616	256.418

Note: Estimates were derived by weighted averages of 24 data points on a daily scale for each sampling month. The IPCC (2013) conversion of CH₄ and N₂O emissions to CO₂ equivalents is 28 and 265, over a 100-year time horizon.

that CH₄ emission during winter ascribed to nearly 40% of the wetland CH₄ emissions to the atmosphere and was thus not negligible. The disparity might be caused by the sampling time. In

our study, we identified both emission and uptake, despite that they were found at different times during the day and that the average CH₄ presented a weak uptake. Morin et al. (2014) might

have missed the negative value of CH₄ fluxes (i.e., uptake, or absorption); continuous observation on a shorter time scale was essential in estimating the diurnal and seasonal variations.

We did not find a significant correlation between the CH₄ fluxes and the air and soil temperatures in different seasons. This result is in accordance with those reported by previous studies (Duan et al., 2005; Chen et al., 2010), indicating that temperature have little effect on the diurnal variation of CH₄ emission. On the other hand, many previous studies have reported that CH₄ flux was strongly correlated with plant biomass (Cheng et al., 2007; Hirota et al., 2007). In our study, the plant biomass was higher in October than that in June (Table 2), yet the CH₄ flux in October was approximately the same as that in June. The reason was that the average temperature in October was lower than that in June. As suggested by Ding and Cai (2007), a low temperature not only reduced the activities of methanogenic bacteria but also left more O₂ transporting into the rhizome or rhizosphere, thereby promoting the oxidization of CH₄. Another explanation might be that the peak of seasonal CH₄ fluxes, which occurred in mid- or late summer (Duan et al., 2005), was missed. The average CH₄ emission was 0.11 mg CH₄ m⁻² h⁻¹ throughout summer to autumn, which was lower than that from Zoige plateau (9.6 mg CH₄ m⁻² h⁻¹). This finding was attributed to the extremely higher total carbon ((226.3±104.1) g/kg) in the Zoige plateau than in our study area ((10.34±1.42) g/kg) (Chen et al., 2010; Xu et al., 2014). However, the average CH₄ fluxes ranged from 0.01 mg to 0.26 mg CH₄ m⁻² h⁻¹ in *S. alterniflora*-covered Jiuduansha wetland (Cheng et al., 2010) and from -0.392 to 0.495 mg CH₄ m⁻² h⁻¹ in *S. salsa*-covered Huanghe (Yellow River) Estuary (Sun et al., 2013), both were approximately the same with our research results. In a word, diurnal variations of CH₄ emissions are characterized by irregular fluctuations.

3.3 Diurnal variations of N₂O fluxes

Diurnal variation in the fluxes of N₂O displayed a single-peak curve in June, declining slightly from 18:00 to 3:00 in the next day (MNF: 9.5±1.7) μg N₂O m⁻² h⁻¹, then increasing gradually to the maximum at 15:00 (86.4 μg N₂O m⁻² h⁻¹), and gradually decreasing until sunset (Fig. 2a, Table 3). N₂O emissions appeared two-peak curves during all the seasons but June. Higher N₂O emissions were found in the morning in October and December, with sub-peaks observed in the afternoon. By contrast, in April, the principal peak was observed in the afternoon at 18:00 (43.8 μg N₂O m⁻² h⁻¹) (Figs 2b–d). The average N₂O emission was 16.8, 21.3, and 23.9 μg N₂O m⁻² h⁻¹ in the daytime and 24.0, 17.8 and 11.8 μg N₂O m⁻² h⁻¹ at night in October, December, April, respectively (Figs 2b–d, Table 3). Similar with CH₄ fluxes, the diurnal N₂O fluxes had significant differences ($p < 0.05$), and the CVs of N₂O emissions were 1.0 in June and 0.6 in October (Table 1).

Previous studies indicated that diurnal variations of N₂O emission varied with space (Maljanen et al., 2002; Allen et al., 2011; Zhang et al., 2015). Our research supplemented that the diurnal variations was diverse on a seasonal scale or different growth stages of *S. alterniflora*. In addition, the mean N₂O emission rate at daytime was five times and twice higher than those during the nighttime in June and April, respectively. This is consistent with the occurrence of low N₂O emission during the nighttime as proposed by Dong et al. (2003) and Zhu et al. (2008). According to Maljanen et al. (2002), this could be attributed to the opening of the stomata in the daytime and closing at night. However, we observed that the N₂O emissions were roughly the same between the daytime and nighttime in October and December (Table 3, Fig. 2). Being the same as the seasonal

changes in CH₄ fluxes, the average N₂O emission in the growing season was higher (29.7 μg N₂O m⁻² h⁻¹ in June and 21.5 μg N₂O m⁻² h⁻¹ in October) than those in the non-growing seasons (17.8 μg N₂O m⁻² h⁻¹ in December and 15.1 μg N₂O m⁻² h⁻¹ in April) (Table 4). The main reason for the variations might be the difference in temperature (Zhu et al., 2008). Significant correlation existed between N₂O emission and air temperature ($r = 0.352$, $p < 0.05$, $n = 32$) on the seasonal scale. The average N₂O flux obtained in this area was lower than that in cultivated and forested organic boreal soils (Maljanen et al., 2002), implying that the invasion of *S. alterniflora* led to less N₂O emission. Elsewhere, along with the finding of Hirota et al. (2007), Zhang et al. (2013) also found that the mean N₂O fluxes during the growing season in *S. alterniflora* was relatively low, i.e., 9.36 μg N₂O m⁻² h⁻¹. Furthermore, in our study, N₂O emission decreased with the growth of *S. alterniflora*. Cheng et al. (2007) and Yu et al. (2012) demonstrated that plants competed with microbes for NO₃⁻ and NH₄⁻ from soil for growth and thus suppressed N₂O emission. Another explanation might be that in October, about the depth of 5 cm waterlogging was observed in the area. Hence, gas diffusion as well as soil O₂ availability decreased, resulting in a reduction of most of the nitrate via denitrification (Davidson, 1993).

In conclusion, if the plant communities were dry, nitrification has a dominant function in regulating N₂O flux, and temperature affected N₂O emission. The daytime N₂O emission was higher than that during the nighttime. However, the diurnal dynamics in N₂O in wet plant communities was different from that in dry environment where denitrification was facilitated.

3.4 Diurnal variations of CO₂ fluxes

In June, the diurnal dynamics in CO₂ fluxes demonstrated a remarkably fluctuating pattern, with a maximum emission of 1 749 mg CO₂ m⁻² h⁻¹ at 9:00 and a minimum of 530.7 mg CO₂ m⁻² h⁻¹ at 6:00 (Fig. 2a). The average CO₂ fluxes were 1 153.5 mg CO₂ m⁻² h⁻¹ in the daytime and 1 002.4 mg CO₂ m⁻² h⁻¹ during the night (Table 3). In October, two peaks were observed at 6:00 (6 912.4 mg CO₂ m⁻² h⁻¹) and 18:00 (3 041.7 mg CO₂ m⁻² h⁻¹). The minimum was 354 mg CO₂ m⁻² h⁻¹ at 12:00 (Fig. 2b). The ratios of daytime and nighttime emissions were 1.2 and 0.4 for June and October, respectively. In December, the highest CO₂ emission (339.8 mg CO₂ m⁻² h⁻¹) was observed at the same time as the lowest CH₄ uptake, and the lowest emission (165.9 mg CO₂ m⁻² h⁻¹) was observed at 21:00 (Figs 2c and d). The average emissions of CO₂ were 311.6 and 712.6 mg CO₂ m⁻² h⁻¹ in the daytime and 192.3 and 303.3 mg CO₂ m⁻² h⁻¹ in the nighttime in December and April, respectively (Table 3).

The average diurnal CO₂ fluxes in October, December, and April exhibited two-peak curves, and the maximum values were observed after sunrise and before sunset. In this time frame, the air temperature changed dramatically, being either above or below the soil temperature (Fig. 1). For example, higher emissions occurred at 6:00 and 18:00 in October and at 9:00 and 18:00 in December, which are in agreement with the diurnal variations of the N₂O fluxes. However, many previous studies have shown that minimum CO₂ emissions occurred in the early morning, and that the highest emissions appeared a few hours later than the highest air temperature (Maljanen et al., 2002; Pei et al., 2003). This finding was inconsistent with our results in June and October but in line with that observed in April. In June, CO₂ emission was lower in the early morning and increased to the maximum value at 9:00, and then it began to fluctuate until 21:00, with a sub-peak of 1 693.7 mg CO₂ m⁻² h⁻¹. CO₂ emission was low at mid-day among the four seasons.

In this study, we observed a higher CO₂ emission in the daytime than that over the night, with ratios of 1.2, 1.6, and 2.3 for June, December, and April, respectively (Table 3). However, in October, the nighttime CO₂ emission was more than twice of that in the daytime (Table 3). Plant respiration was similar every night in drained and flooded conditions (Miyata et al., 2000). Hence, the difference might be explained by the stomata opening and closure during the daytime and nighttime, respectively. As mentioned above, in the water logged environment, CH₄ was easily oxidized into CO₂ (Smith et al., 2003) and then emitted to the atmosphere when the stomatal conductance was closed. In addition, the mean nighttime emissions were 1 002.4, 2 830.8, 192.3, and 303.3 mg CO₂ m⁻² h⁻¹ for June, October, December, and April, respectively (Tables 3 and 4) suggesting that CO₂ emission increased with the growth of *S. alterniflora*, and so did the MF of CO₂.

Previous studies have reported that solar energy input and associated temperature changes were the most important factors that control the diurnal variations of CO₂ emission (Nieveen et al., 1998; Maljanen et al., 2002; Pei et al., 2003; Zhang et al., 2015).

In our current study, solar energy was controlled by measurement with static opaque chamber. No obvious correlation was found between CO₂ emission and air and soil temperatures (Table 5). This is because that several interacting mechanisms were involved in determining the diurnal variation in CO₂ net flux (Davidson et al., 2000). Hence, the effect of temperature was easily weakened and offset by other factors. Parkin and Kaspar (2003) stated that CO₂ flux-temperature relationships might be more complex in the field, where temperatures varied with time and soil depth, than those conducted in the laboratory. However, the highest air temperature and CO₂ emission occurred approximately at the same time (9:00), as revealed in the study of Hirota et al. (2007) and Morris and Whiting (1986). Our results also indirectly proved that ecosystem respiration in our research was higher (1 078 mg CO₂ m⁻² h⁻¹) than soil respiration during summer season (725 mg CO₂ m⁻² h⁻¹, here only the peak value was obtained) as reported by Hirota et al. (2007).

3.5 GWP and TFD

The GHG emissions during the four seasons are presented in

Table 5. The relationship between CH₄, N₂O, and CO₂ and air temperature and soil temperature

	Growing season				Non-growing season				The annual	
	Jun.		Oct.		Dec.		Apr.		A	S
	A	S	A	S	A	S	A	S	A	S
CH ₄	-0.324	N	0.126	0.302	-0.343	0.121	-0.289	-0.572	0.196	0.048
N ₂ O	0.854**	N	-0.510	-0.477	0.219	-0.038	0.240	0.454	0.352*	-0.009
CO ₂	0.433	N	-0.495	-0.657	0.655	0.571	0.545	0.295	0.185	0.286

Note: **p*<0.05; ***p*<0.01. A represents air temperature, S soil temperature, and N not measured. The relationship during the growing season and non-growing season was based on *n*=8; the relationship during the annual flux was based on *n*=32 for air temperature and *n*=24 for soil temperature.

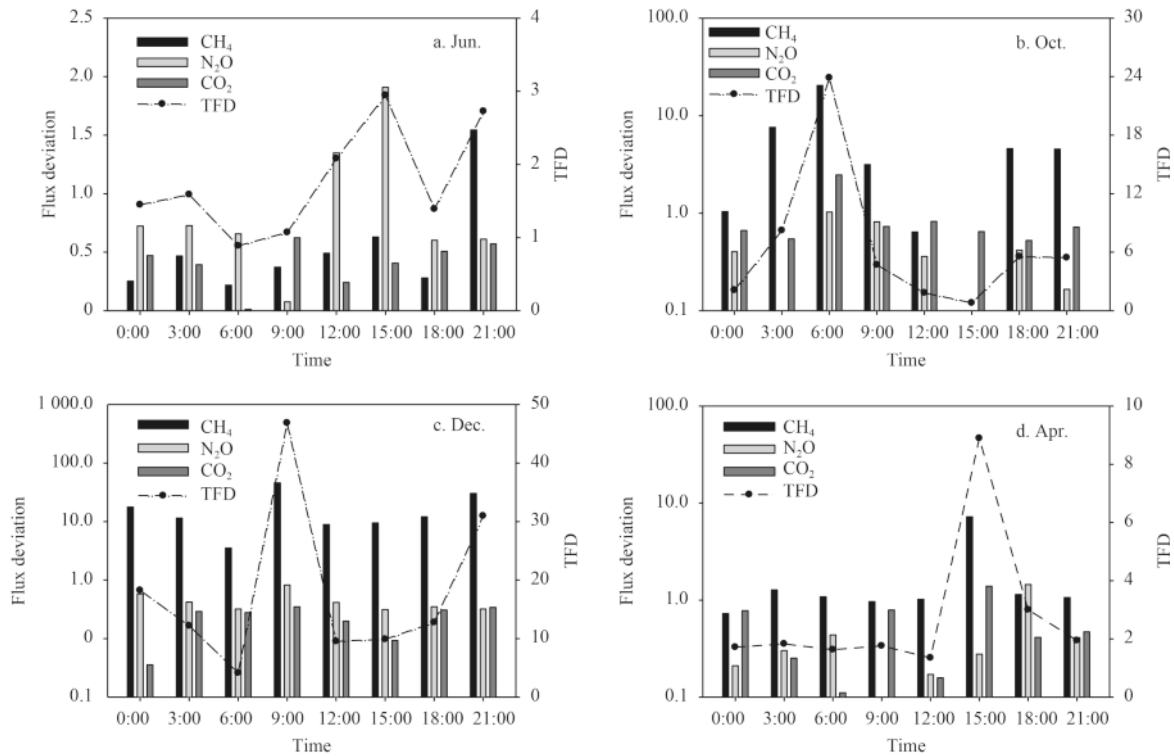


Fig. 3. Flux deviations of CH₄, N₂O, and CO₂ and total flux deviations (TFD) in *S. alterniflora*-covered flats during different seasons in Yangcheng coastal saline wetland. a. June (summer) 2013, b. October (autumn) 2013, c. December (winter) 2013, and d. April (spring) 2014.

2014. $TFD = FD(CH_4) + FD(N_2O) + FD(CO_2)$, take CH₄ for example, $FD(CH_4) = \sum_{i=1}^{n=8} \sqrt{\left(\frac{MF_{CH_4} - F_{i-CH_4}}{MF_{CH_4}}\right)^2}$.

Table 3. Compared with N₂O fluxes, which were emitted throughout the observation period, CH₄ was emitted in the growing season and functioned as a sink in the non-growing season. When CH₄ and N₂O emissions were expressed as CO₂-e emissions only (without the consideration of CO₂ emissions), the contribution of N₂O was high in June, followed by October during the growing season, which were slightly higher than those during the non-growing season. The contribution of CH₄ was the same as that made by N₂O, whereas CH₄ had a net sink in the non-growing season (Table 4). In the growing season, N₂O comprised 64.1% to 92.1% of the net CO₂-e measured.

TFD was used to demonstrate the deviation between the fluxes at different times of a day and *MF*. As suggested in Fig. 3, in June and December, the optimal measurement time was calculated at 6:00 in the early morning, whereas in April and October, the period from 12:00 to 15:00 was ideal for sampling. In general, the *TFD* in April and June were later than those in October and April.

The same trend was observed for seasonal changes of *TDF*, *MF*, and *GWP*, with higher values in the growing season compared with the non-growing season. Except CO₂, the *TDF*, *TFD*, and *GWP* of N₂O and CH₄ were all higher in June than those in October. N₂O emissions contribute dominantly to global warming potential if CO₂ was excluded from the *GWP* (Table 4, Fig. 3). Large diurnal variations also indicated that any intermittent observations at low - measurement frequency of at most once per day or even lower was likely to cause an over- or underestimation of the emission (Wang et al., 2005). Our results provided as an available reference for further investigation, since we collected the data at the optimal sampling time for different seasons (Fig. 3).

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