

SPATIAL VARIABILITY IN GREENHOUSE GAS FLUXES OF A TEMPERATE FRESHWATER MARSH IN CHINA: EFFECTS OF SOIL MOISTURE, ANIMAL ACTIVITIES AND LAND USE

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Abstract. The greenhouse gas (CH₄, N₂O and CO₂) fluxes from Shengjin Lake Marsh (SLM) were *in situ* investigated at the undisturbed sites and the disturbed sites by animal colonization and human activities. Overall the CH₄ emissions from the wet sites were one order of magnitude higher than those from the mesic or dry sites, whereas higher N₂O emissions and net CO₂ uptake occurred at dry sites rather than at mesic and wet sites. Soil moisture was the predominant factor controlling the spatial variability in marsh CH₄ and N₂O fluxes and NEE at undisturbed sites. Mean CH₄ fluxes from migrating water bird and poultry active areas showed no statistically significant differences from the undisturbed sites, but their activities significantly increased N₂O emissions and decreased marsh CO₂ sink, with the highest N₂O fluxes (mean 49.5±11.9 μg N₂O m⁻² h⁻¹) in poultry active areas. The conversion of the marsh into agricultural field and landfill did not significantly change CH₄ and N₂O fluxes, but significantly decreased CO₂ sink. Our results revealed that the accurate evaluation of GHG budget for the SLM and other similar temperate wetlands should involve soil water regimes, water bird colonization and human-induced influences.

Keywords: CH₄ flux, N₂O flux, NEE, Shengjin Lake Marsh, temperate wetland

Introduction

Atmospheric carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) are key radiatively active greenhouse gases (GHGs) (Ravishankara et al., 2009). Natural wetlands play a critical role in the global GHG budgets due to the storage of a large amount of carbon and nitrogen (Post et al., 1982), the high temperature sensitivity of microbial respiration, and nitrogen mineralization (Davidson and Janssens, 2006; Song et al., 2009). The wetlands are one of important sources for atmospheric GHGs (Jungkunst and Fiedler, 2007; Marani and Alvala, 2007; Song et al., 2009). For example, natural wetlands present the largest single CH₄ source, accounting for more than 75% of natural CH₄ source and more than 20% of the global CH₄ source (Kirschke et al., 2013). Global wetland ecosystems also contribute considerably to N₂O budget although sometimes the N₂O emissions from the wetlands are very low (Martikainen et al., 1993; Xu et al., 2008). Therefore, it is necessary to conduct the research about GHG fluxes from the wetlands in different climatic zones.

At present, net GHG fluxes have been extensively measured at a number of sites of global wetlands to better understand the relationships between GHG fluxes and environmental parameters (Jungkunst and Fiedler, 2007; Marani and Alvala, 2007; Song et al., 2009). China is home to the world's fourth largest wetland area (Wang et al., 2012), and *in situ* GHG flux observations have covered the wetlands in Northeast China (Ding et al., 2004; Ding and Cai, 2007; Song et al., 2009), the Tibetan Plateau (Chen et al., 2008; Song et al., 2015; Wei et al., 2015), Inner Mongolia (Duan et al., 2005; Wang et al., 2009), coastal salt marshes (Song and Liu, 2016), and the Middle-Lower Yangtze Floodplain (MLYP) wetlands including the freshwater marshes in Poyang Lake and Taihu Lake (Xing et al., 2005; Wang et al., 2006; Hu et al., 2016). Observations suggest that the wetlands showed the micro- and macro-scale spatial pattern of GHG release (Kato et al., 2011; Song and Liu, 2016; Wei and Wang, 2017), and soil physicochemical properties, soil microbial community structure, vegetation coverage and warming climate are important factors affecting wetland GHG fluxes (Johansson et al., 2006; Wei and Wang, 2017). The MLYP is characterized by dense human population and rapid economic development, which both impose unprecedented pressure on the wetland ecosystems, and has caused serious wetland degradation (Zhou et al., 2010). In addition, the MLYP wetland is an important wintering habitat and stopover site for migrating waterbirds on the East Asia-Australia Flyway (Fang et al., 2006). Environmental variables, animal colonization and human activities might lead to spatial variability in GHG emissions from the MLYP wetland. A better understanding of the spatial patterns in the GHG fluxes and their controlling factors is crucial to accurately estimate the regional and global GHG budgets from wetlands (Song et al., 2009; Kirschke et al., 2013; Wei and Wang, 2017). Nevertheless, the data about GHG fluxes are still lacking in the MLYP wetlands, and especially effects of animal colonization and human activities on GHG fluxes are highly neglected.

The Shengjin Lake Marsh (SLM), as a natural freshwater wetland, is located in the MLYP with temperate monsoon climate and an evident distinction between rainy and dry seasons in the precipitation. Part of the marsh is often converted into farmlands including paddy fields or wheat lands with the drawdown of water level. This marsh also provides an important natural colony for some migrant waterbirds to overwinter and breed, and a natural raising area for the poultry including ducks and geese (Lu and Liu, 2011). The natural disturbances due to flood and drought, animal colonization and human activities have intensively affected the local environment (Yang et al., 2015). However, effects of these natural and anthropogenic factors on GHG fluxes to date have not been investigated in the SLM. During summer and winter in 2013, the SLM was selected as study area, and CH₄, N₂O and CO₂ fluxes were simultaneously observed at the upland and lowland marsh areas, and the marsh sites disturbed by animal colonization and land use. Our objectives were (1) to study spatial variability in GHG fluxes from the marsh; (2) to investigate the effects of soil moisture on GHG fluxes; (3) to examine the effects of animal activities and land use on GHG fluxes. This is an important attempt to reasonably evaluate the importance of soil moisture, animal activities and land use in the GHG budgets in the MLYP wetland.

Materials and methods

Study area

The Shengjin Lake Wetland National Nature Reserve (116° 55' E-117°15' E, 30° 15' N-30° 30' N) is located in the Middle-Lower Yangtze Plain (Fig. 1). The wetland covers an area of approximately 133 km² in the rain season and only 33 km² in the dry season. This area has a temperate monsoon climate with a hot and humid summer, and a cold and dry winter; its mean annual air temperature and precipitation is about 16.1°C and about 1600 mm, respectively (Xu et al., 2008). Topographically the Shengjin Lake can be divided into three parts: the upper lake, the middle lake and the lower lake. The marsh around the lake has diverse vegetation types including *Artemisia lavandulaefolia* DC, *Artemisia scoparia* waldst and *Pinus massoniana* (Lu and Liu, 2011; Yang et al., 2015). It is a natural colony for over 100 species of migratory birds to overwinter and breed (Lu and Liu, 2011). The common species of waterbirds include bean goose *Anser fabialis*, greater white-fronted goose *Anser albifrons*, lesser white-fronted goose *Anser erythropus*, swan goose *Anser cygnoides* and tundra swan *Cygnus columbianus bewickii* (Zhang et al., 2015). Overall the Shengjin Lake Marsh (SLM) has been experiencing the disturbance from human activities, poultry raising and natural disturbances from waterbirds, drought and flooding (Jiang et al., 2007; Lu and Liu, 2011; Fox et al., 2013).

More information was given in S1 for Electronic Appendix.

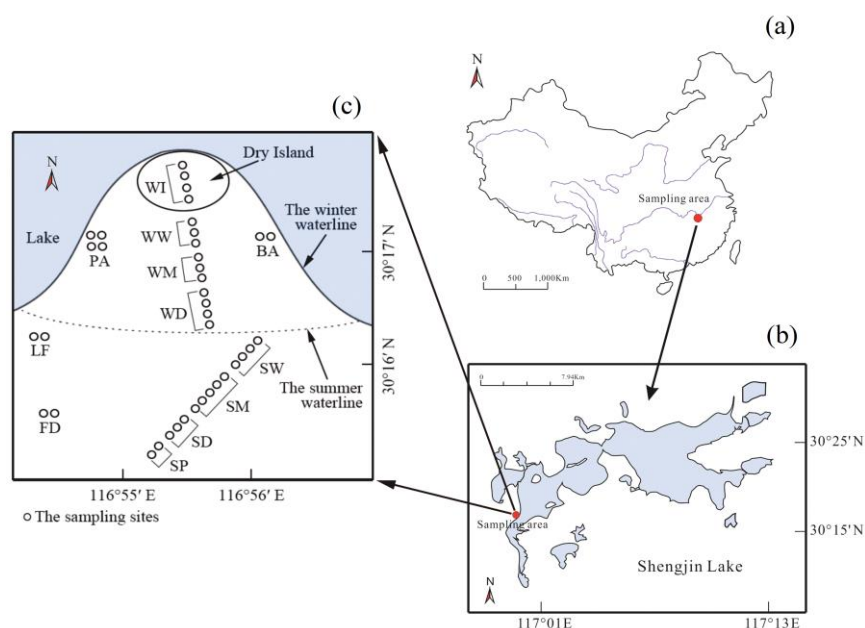


Figure 1. (a) The red dot indicates location of the investigation area in China. (b) The topographic feature of Shengjin Lake and the red dot indicates location of the investigation area in Shengjin Lake. (c) The location of the investigation sites for the SLM in the summer and winter. SD, SM, SW and SP represent the dry, mesic and wet marsh sites, and paddy field sites in the summer, respectively; WD, WM, WW and WI represent the dry, mesic and wet marsh sites, and the island marsh sites, respectively; BA, PA, LF and FD represents water bird active area, poultry active area, landfill and farmland, respectively. In the summer, the lake water level rose up to the highest, and the marsh formed along the summer waterline. In the winter, a large flooded land area was exposed with the decrease in the lake water level and the marsh formed along the winter waterline

Investigation sites and experimental design

To test effects of soil moisture, waterbird colonization and human activities on the spatial variability in GHG fluxes from the SLM, a series of the flux observation sites were established within the following three areas located on the western shore of the upper lake as illustrated in *Fig. 1*:

(i) The upland marsh area: In summer, the lake water level rises up to the highest along the shore. In July 2013 (From July 1st to July 30th), a total of 14 stations (sj1-1 to sj1-14) for summer GHG flux observations were set up from the wet to dry marsh areas along a transect. These stations were further divided into four types of sites according to soil water regime: (a) wet site (SW), which were near the lake with almost no vegetation; (b) mesic site (SM) with few vegetation; (c) dry site (SD) with the extensive vegetation; and (d) paddy site (SP), which was located at the uppermost locality of the transect with changing water levels. Two repetitions of GHG flux measurements were made at each station.

(ii) The lowland marsh area: A large flooded land area was exposed in winter with the drawdown of water level. In December 2013 (From December 1st to December 30th), we selected a long transect from the upland to the lowland, and then to a small island along the shore. A total of 14 stations (sj2-1 to sj2-14) along the transect were set up for GHG flux measurements. These stations were divided into four types of sites as follows: (a) dry marsh site (WD); (b) mesic marsh site (WM); (c) wet marsh site (WW); (d) dry island marsh site (WI). Two repetitions were made at each station.

(iii) The marsh sites disturbed by animal activities and land use: In December 2013, a total of 10 stations were set up in the disturbed areas for GHG flux measurements. These stations were divided into four types of sites: waterbird active site (BA, two stations), poultry active site (PA, four stations), landfill (LF, two stations) and farmland (FD, two stations). Two repetitions were made at each station. In *Electronic Appendix*, more detailed information was given in S2, and soil physiochemical properties at all the stations were summarized in *Table S1* and *Table S2*.

In-situ GHG flux measurements

The GHG fluxes from all the stations were measured in the SLM during two sampling campaigns (July, 2013 in the summer, and December, 2013 in the winter) using a static chamber technique (Hutchinson and Mosier, 1981; Zhu et al., 2014a,b). Open-bottomed clear plexiglass chambers (50 cm×50 cm×50 cm) were placed on the PVC collars installed at the measurement sites. The collars enclosed an area of about 0.25 m² and were inserted into the soils to a depth of about 5 cm. The use of flux collars ensures that flux chambers are well sealed since the chambers fit into a water-filled notch in the collars. The average chamber height was 45 cm above the ground, which met the minimum required without influencing gas diffusion patterns under normal atmospheric pressure (Hutchinson and Mosier, 1981).

During gas flux measurements, upon enclosure of the cover chambers, headspace gas samples were collected at 0, 10, 20 and 30 min intervals with a double ended needle connected to pre-vacuated glass vials (18 mL) with butyl rubber septa. For each gas flux measurement, a total of four samples were withdrawn from each chamber after enclosure. Internal air temperature inside the chambers was simultaneously measured using a thermometer installed on the chamber. Ground temperature (GT) and soil moisture (Mc) were measured before and after the gas sampling. The order of

measurements was varied to ensure that the measuring time did not bias the results, resulting in two replicate measurements per station.

Net ecosystem exchange (NEE), defined as net ecosystem CO₂ flux under light conditions, was measured by the change in CO₂ concentrations in transparent chambers (Ström et al., 2007). In summer 2013, immediately following NEE measurements, ecosystem respiration (ER) was measured by the same procedure after darkening of the chamber covered by three layers of thick black cloth. Marsh ER includes soil and vegetation respiration, and photosynthesis was calculated as the difference between NEE and dark respiration (Ström et al., 2007).

Determination of GHG concentrations and flux calculation

The methods of analyzing GHG concentrations and flux calculation are described in detail in our previous papers (Zhu et al., 2014a,b; Bao et al., 2018a,b). In brief, N₂O, CH₄ and CO₂ concentrations were analyzed using gas chromatography equipped with a ⁶³Ni electron capture detector, a flame ionization detector and a thermal conductivity detector, respectively (GC-HP5890 II, USA; Shimadzu GC-12A, Japan; Shimadzu GC-14B, Japan). Net GHG fluxes were calculated using a linear least squares fit to four points in the time series of concentrations with an average chamber temperature. The least squares regression lines “headspace GHG concentrations versus time” were first visually inspected for abrupt changes in the direction of the flux, resulting from disturbances such as the leakage of the chamber or disturbances of soils during sampling. The correlation coefficient $R^2 > 0.95$ for linear regression was used as a quality check for the measurement. If the fluxes are low, the random error of the measurement can be larger than the change in GHG concentrations between two sampling points. Discarding these fluxes based on low R^2 values would lead to an overestimation of the overall fluxes. Some low fluxes were included regardless of their R^2 values. For the gas fluxes, positive values indicate net emission to the atmosphere, and negative values indicate net uptake from the atmosphere. More information was given in S3 for *Electronic Appendix*.

Environmental variables and general soil properties

Altogether 41 soil samples (3 samples were taken at each station except sj1-1 only 2 samples) in summer and 48 soil samples (2 samples were taken at each station) in winter were taken along with the same stations where GHG fluxes were measured. All the soil samples were air-dried, milled and screened using the 200 mesh sifter for the detection. Soil gravimetric moisture content (M_C) was determined by drying the soil at 105°C for 12 h. M_C was calculated as: $M_C = \frac{\text{weight of the lost water}}{\text{dry soil weight}} \times 100\%$. The pH was determined in distilled water and in 1M KCl solution (soil: solution ratio=1:3). Total organic carbon (TOC) was analyzed from the dry soil by the potassium dichromate volumetric method with an analytical error of 2.5%. Total carbon (TC), total nitrogen (TN) and total sulfur (TS) were determined using vario MACRO CHNS analyzer. The NH₄⁺-N and NO₃⁻-N were determined by an ion-selective electrode method with an analytical error of <5.0%, and total phosphorus (TP) was determined by ultraviolet visible spectrometry (UV-2450, Shimadzu, Japan) with an analytical error of <2.0% (Zhu et al., 2009).

Statistical analysis

Microsoft Excel 2013 and SPSS 20.0 are used to make the statistical analysis of the data under the Windows 10 environment. For each station, we used standard error (SE) to estimate the uncertainty of the individual fluxes. The individual fluxes and their associated uncertainty were then averaged for each type of marsh site. All the data for GHG fluxes were expressed as mean $\pm$ SE. We used the coefficient of variance (CV) of those flux as a measure of intra-site spatial variability. The statistically significant differences between the means for each type of sites in the upland, lowland and disturbed marsh areas were tested using Fisher's Least Significant Difference (LSD, $P < 0.05$) tests. We used the Principal Component Analysis (PCA) as a multivariate exploratory technique to explain the main factors affecting spatial variability in GHG emissions at the undisturbed and disturbed sites. To find factors causing spatial variability in the fluxes, the correlations of GHG fluxes with environmental variables were tested separately for all the types of sites using Spearman's rank order correlation. A univariate exponential function model (Eq.1) was fitted for the relationship between ER and soil temperature:

$$ER = \beta_0 e^{\beta_1 T} \quad (\text{Eq.1})$$

where ER is ecosystem respiration ($\text{mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$), T is mean soil temperature ($^{\circ}\text{C}$), and β_0 and β_1 are the constants fitted with the least squares technique. The Q_{10} value was calculated as (Eq.2):

$$Q_{10} = e^{10\beta_1} \quad (\text{Eq.2})$$

In all analyses where $p < 0.05$, the factor tested and the relationship were considered statistically significant. Although our data might represent a rough estimate of GHG fluxes, we still used the GWP (Global Warming Potential) as a conversion factor to evaluate the impact of the SLM on the greenhouse effect. Based on a 100-year time frame, the GWPs of CH_4 and N_2O are, respectively, 25 and 298 times higher than that of CO_2 (Ravishankara et al., 2009). The net GWP were calculated by taking mass factors, 1 for CO_2 , 25 for CH_4 , and 298 for N_2O .

Results

Environmental variables in the marsh

The soils at the upland marsh transect were slightly acid with the pH of 5.2-6.1, and soil moisture decreased from the lowland to the upland marsh with the range of 17%-59% (Table S1 and Table S2). Ground temperatures showed no significant differences between four types of sites in summer or in winter (Fig. 2). TC, TOC and TN levels in the soils showed no significant differences. The TC and TOC levels were very close to each other (average 1.24% and 1.13%), and they showed a significant correlation ($R = 0.941$), indicating that soil carbon in the marsh mainly existed as organic carbon. Soil organic carbon content (average 2.62%) at the lowland marsh was almost twice higher than that at the upland marsh (average 1.13%). Soil NO_3^- -N contents were one to two orders of magnitude higher than NH_4^+ -N contents at upland or lowland

marsh transects. Soil NO_3^- -N contents in landfill, poultry and waterbird active areas and farmland were evidently higher than those in non-disturbed marsh areas, and extremely high NO_3^- -N occurred in poultry active soils ($1143.4 \mu\text{g g}^{-1}$). In addition, soil moisture showed evident differences with the range of 19%-73% between the types of marsh sites due to effects of animal activities and land use.

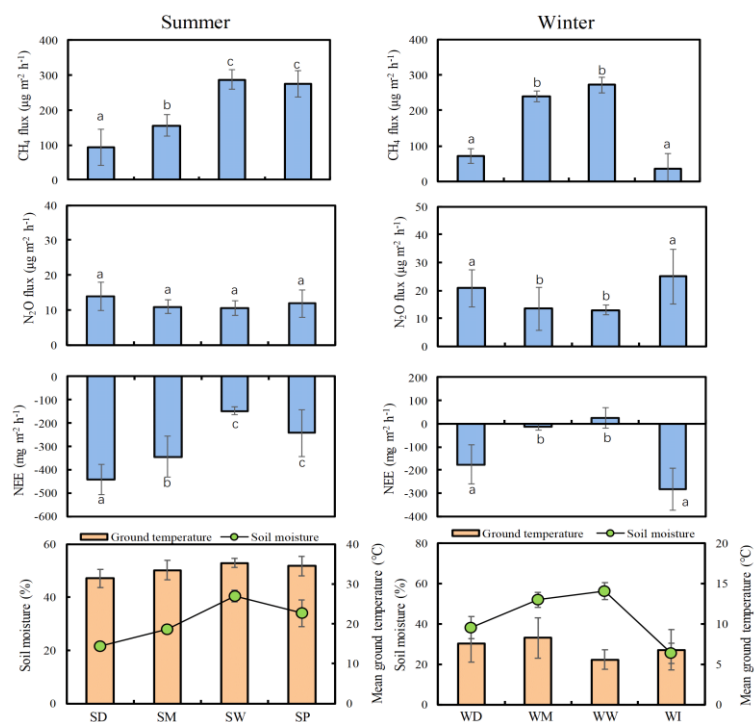


Figure 2. Spatial variability in CH_4 , N_2O and CO_2 fluxes from the SLM transects during the summer and winter. The given data are the mean values at each site in the marsh, and the vertical bars indicate standard errors of GHG fluxes. (a) In the summer, SD ($n=6$), SM ($n=10$), SW ($n=8$) and SP ($n=4$) represent the dry, mesic and wet marsh sites, and paddy field sites, respectively; (b) In the wintertime, WD ($n=8$), WM ($n=6$), WW ($n=6$) and WI ($n=8$) represent the dry, mesic and wet marsh sites, and the island marsh sites, respectively. The different lowercase letters (a, b, c) indicate statistically significant differences between the mean values (Fisher's LSD, $P < 0.05$)

CH_4 fluxes

The wet marsh sites SW and WW showed strong CH_4 emissions with the mean fluxes of $287.3 \pm 51.1 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$, and $271.2 \pm 21.1 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$, respectively. Dry marsh sites SD and WD showed net weak CH_4 emissions with the mean rates of $93.9 \pm 27.8 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ and $70.1 \pm 20.7 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$, one order of magnitude lower than those from the mesic and wet sites, whereas the lowest CH_4 emission occurred at the dry island site WI (Fig. 2). The paddy site SP showed strong CH_4 emission ($275.8 \pm 37.7 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$), comparable to the sites SW and WW, but significantly higher than those at the sites SM and SD (Table 1). Overall the fluxes from waterbird active area ($148.3 \pm 51.5 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$) and landfill ($98.8 \pm 28.9 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$) were higher than those from poultry active area ($64.4 \pm 44.3 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$) and farmland ($20.0 \pm 105.0 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$) although their mean fluxes showed no statistical significant differences (Fig. 3).

Table 1. Comparisons of CH₄, N₂O and CO₂ fluxes at the different types of sites in Shengjin Lake Marsh

Marsh site types	CH ₄ flux (μg CH ₄ m ⁻² h ⁻¹)			N ₂ O flux (μg N ₂ O m ⁻² h ⁻¹)			NEE (mg CO ₂ m ⁻² h ⁻¹)			ER (mg CO ₂ m ⁻² h ⁻¹)		
	Range	Mean ± SE	CV (%)	Range	Mean ± SE	CV (%)	Range	Mean ± SE	CV (%)	Range	Mean ± SE	CV (%)
Summertime, July, 2013												
SW	152.0-773.0	287.3±51.1a	17.8	1.5-18.3	10.6±4.1a	38.7	-270.6-28.3	-148.5±65.0a	43.8	218.9-1531.9	694.2±420.1a	60.5
SM	10.2-399.8	155.9±31.5b	20.2	6.4-16.5	11.0±2.0a	18.2	-552.5 to -42.6	-345.9±88.1b	25.5	15.7-1132.9	674.9±210.8a	31.2
SD	62.6-149.3	93.9±27.8c	29.6	10.3-17.7	13.9±2.1a	15.1	-473.8 to -415.0	-443.6±17.0c	3.8	926.9-1305.5	1106.9±109.7b	9.9
SP	139.6-412.0	275.8±37.7a	13.7	8.1-15.7	11.9±3.8a	31.9	-472.2 to -12.8	-242.5±101.2a	41.7	887.2-1200.1	1043.7±156.5b	15.0
Comprehensive	10.2-773.0	197.3±205.5	104.2	1.5-18.3	11.6±4.9	42.2	-552.5-28.3	-295.7±192.9	65.2	15.7-1305.5	835.8±446.1	53.4
Wintertime, December, 2013												
WW	127.4-403.8	271.2±21.1b	7.8	10.2-16.2	13.0±1.7b	13.1	-406.0 to -104.2	-24.1±43.8b	1.8			
WM	212.3-265.5	240.3±15.4b	6.4	-1.8-21.9	13.4±7.6b	56.7	-39.4-8.5	-14.5±13.9b	96.0			
WD	20.9-110.5	70.1±20.7a	29.5	9.8-38.0	20.8±6.7a	32.2	-323.8-48.8	-176.1±84.2a	47.8			
WI	-35.5-104.8	36.4±40.5a	111.3	14.1-35.2	25.1±9.8a	39.0	-44.3-151.5	-284.7±90.1a	31.6			
Comprehensive	-35.5-403.8	108.3±191.1	176.5	-1.8-38.0	18.8±10.9	58.0	-406.0-151.5	-129.6±173.0	133.5			

The measured GHG fluxes at marsh site types with the same suffix letter (a, b or c) are not significantly different from one another (LSD, P< 0.05). Marsh ER was not observed in 2013 winter

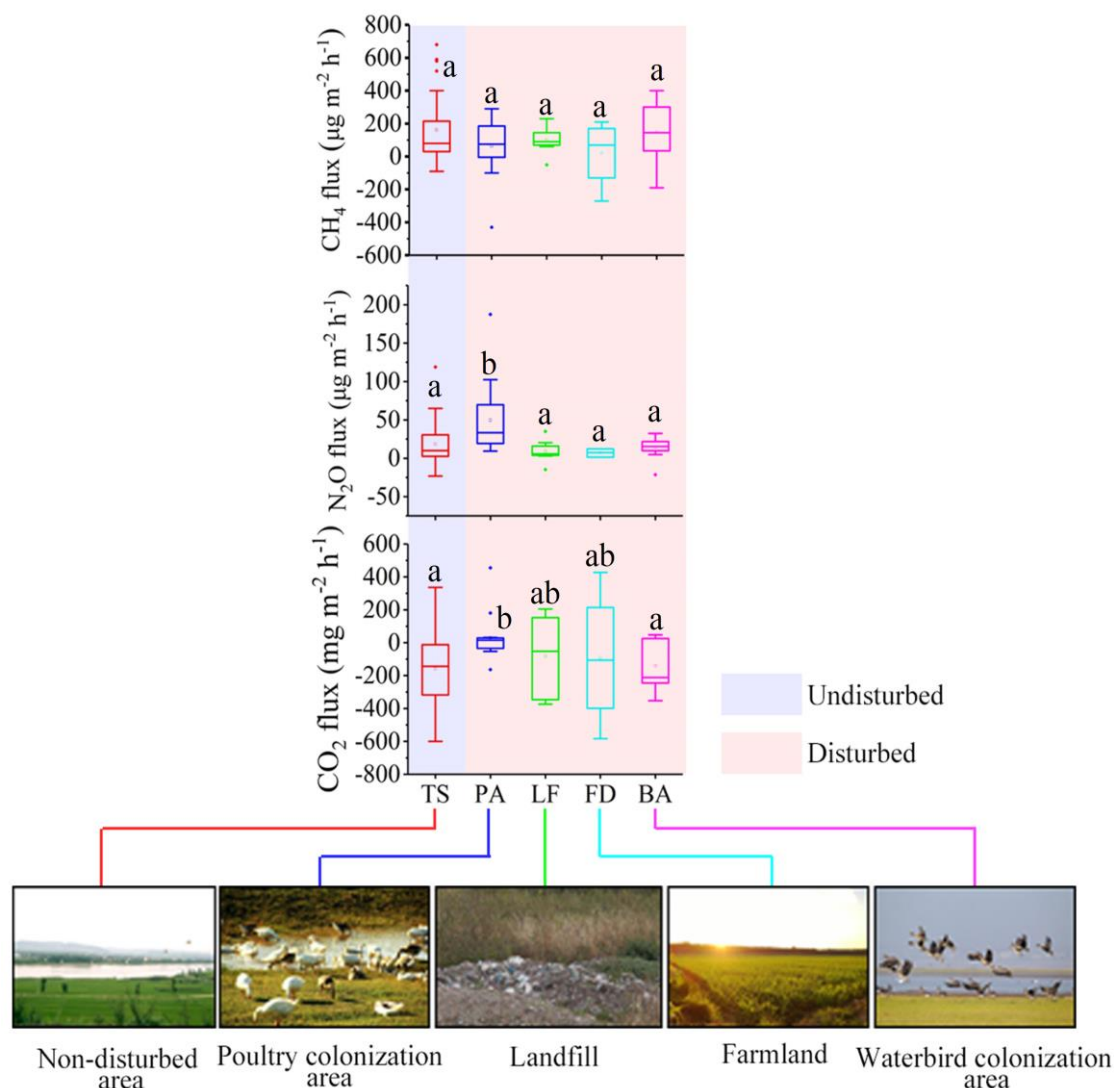


Figure 3. Comparisons of CH₄, N₂O and CO₂ fluxes from different non-disturbed and disturbed marsh areas. TS (n=56), PA (n=8), LF (n=4), FD (n=4) and BA (n=4) represent non-disturbed site, poultry colonization area, landfill, farmland, and waterbird colonization area, respectively. The different lowercase letters (a, b) indicate statistically significant differences between the mean fluxes (Fisher's LSD, P < 0.05)

The PCA analysis showed that the environmental variables in the first two components explained 71.0% and 87.2% of the cumulative variance of the CH₄ fluxes at the undisturbed and disturbed marsh sites, respectively, and soil moisture was the predominant factor affecting spatial variability in CH₄ emission (Fig. 4). The CH₄ fluxes showed a significant positive correlation with soil moisture at the undisturbed or disturbed marsh sites. When the data at all the observation sites were combined, CH₄ fluxes showed a strong positive correlation (P=0.000) with soil moisture (Table 2). The TN, NH₄⁺-N did not significantly affect CH₄ emissions at the undisturbed sites, whereas they showed a significant positive correlation with CH₄ emissions at the disturbed marsh sites.

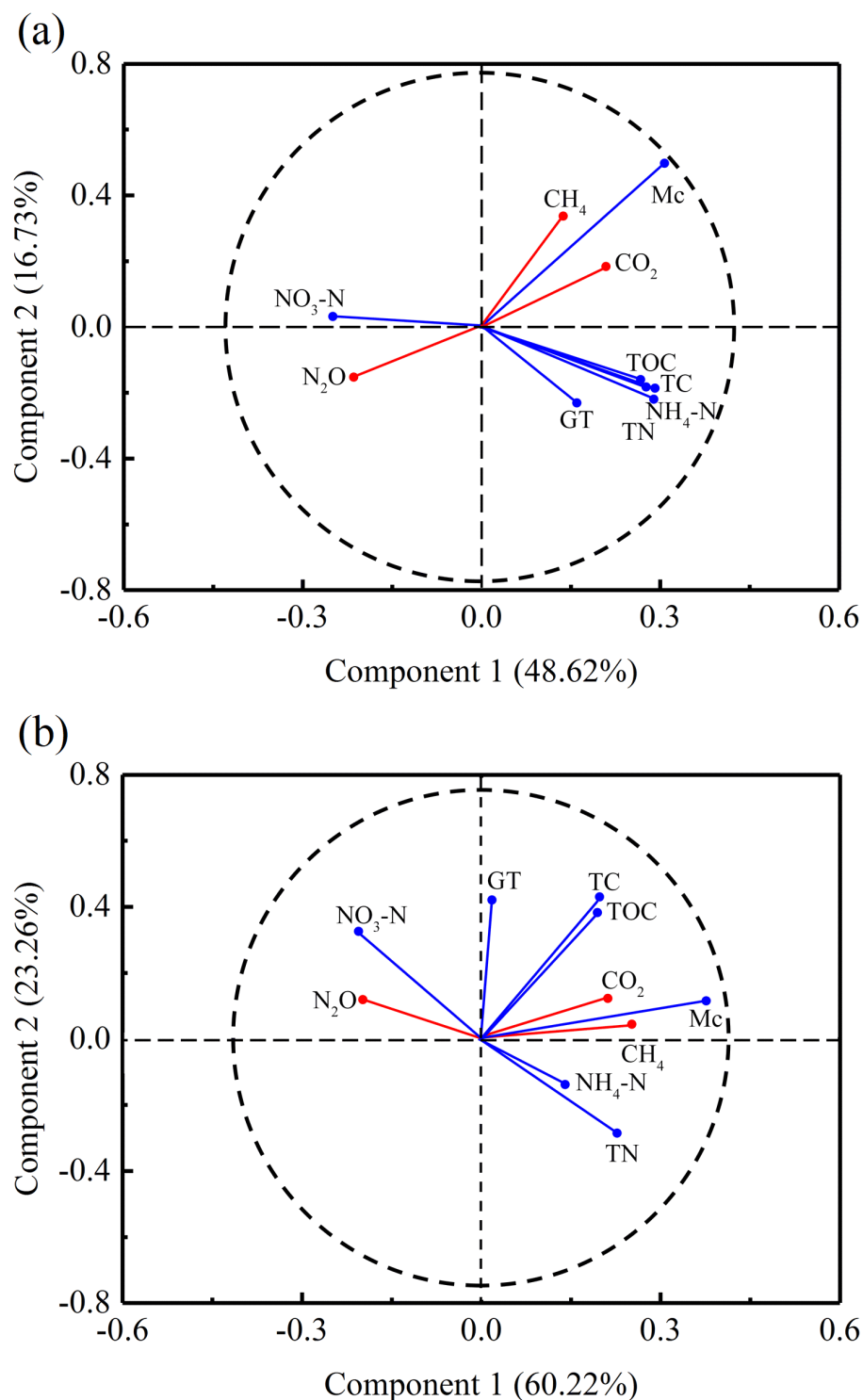


Figure 4. Correlation bi-plot between GHG fluxes and environmental variables in non-disturbed (a) and disturbed (b) marsh areas based upon the standardized principal component analysis. The first two dimensions explained 65% and 83% of the variation for non-disturbed and disturbed marsh areas, respectively. Mc, TOC, TN, TC and GT indicate soil moisture, total organic carbon, total nitrogen, total carbon and ground temperature, respectively

Table 2. Correlations between marsh greenhouse gas fluxes and environmental parameters during the observation period. Significant statistics ($P < 0.05$) are highlighted in bold type

	Undisturbed marsh sites (n=69)			Disturbed marsh sites (n=20)			Overall (n=89)		
	CH ₄ Flux ($\mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$)	N ₂ O Flux ($\mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$)	CO ₂ Flux ($\text{mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$)	CH ₄ Flux ($\mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$)	N ₂ O Flux ($\mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$)	CO ₂ Flux ($\text{mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$)	CH ₄ Flux ($\mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$)	N ₂ O Flux ($\mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$)	CO ₂ Flux ($\text{mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$)
Mc (%)	0.449	-0.656	0.258	0.556	-0.578	0.501	0.641	-0.704	0.244
	P=0.000	P=0.000	P=0.032	P=0.011	P=0.008	P=0.025	P=0.000	P=0.000	P=0.021
TOC (%)	0.053	-0.190	0.190	0.065	-0.449	0.171	0.074	-0.254	0.114
	P=0.664	P=0.235	P=0.235	P=0.787	P=0.047	P=0.472	P=0.492	P=0.038	P=0.289
TN (%)	0.018	-0.124	0.091	0.459	-0.509	0.186	0.313	-0.244	0.124
	P=0.880	P=0.439	P=0.456	P=0.042	P=0.022	P=0.433	P=0.046	P=0.021	P=0.439
TC (%)	0.036	-0.141	0.134	0.050	-0.502	0.217	0.043	-0.223	0.138
	P=0.825	P=0.379	P=0.403	P=0.835	P=0.024	P=0.359	P=0.689	P=0.036	P=0.197
NH ₄ ⁺ -N ($\mu\text{g g}^{-1}$)	0.021	-0.128	0.124	0.484	-0.531	0.227	0.224	-0.313	0.206
	P=0.862	P=0.283	P=0.439	P=0.031	P=0.016	P=0.336	P=0.036	P=0.046	P=0.196
NO ₃ ⁻ -N ($\mu\text{g g}^{-1}$)	-0.025	0.237	-0.128	-0.208	0.752	-0.291	-0.089	0.641	-0.223
	P=0.841	P=0.049	P=0.293	P=0.380	P=0.000	P=0.214	P=0.581	P=0.000	P=0.036
GT (°C)	0.010	-0.089	0.085	0.202	-0.186	0.057	0.124	-0.114	0.094
	P=0.948	P=0.581	P=0.492	P=0.394	P=0.433	P=0.812	P=0.439	P=0.289	P=0.383

Undisturbed, disturbed sites indicate regular transect sites and the marsh sites disturbed by waterbird colonization and human activities, respectively. Mc, TOC, TN, TC and GT indicate soil moisture, total organic carbon, total nitrogen, total carbon and ground temperature, respectively

N₂O fluxes

The dry marsh sites SD and WD showed strong N₂O emissions with the mean fluxes of $13.9 \pm 2.1 \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$ and $20.8 \pm 6.7 \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$, respectively, whereas the mesic sites SM and WM and wet sites SW and WW presented weak N₂O emissions (Fig. 2). The mean fluxes from SM ($11.0 \pm 2.0 \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$) and WM ($13.4 \pm 7.6 \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$) were comparable to those from SW ($10.6 \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$) and WW ($13.0 \pm 1.7 \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$). High emission ($25.1 \pm 9.8 \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$) occurred at the site WI, corresponding to the lowest soil moisture (Fig. 2). Overall the highest N₂O fluxes (mean $49.5 \pm 11.9 \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$) and slightly enhanced fluxes (mean $14.1 \pm 3.9 \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$) occurred in poultry and waterbird active areas, respectively (Fig. 3). The landfill site LF (mean $8.9 \pm 5.1 \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$) and farmland site FD (mean $5.2 \pm 2.8 \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$) showed relatively low N₂O fluxes, comparable to the undisturbed sites (mean $8.7 \pm 4.2 \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$).

The PCA analysis showed that environmental variables in the first two components explained 72.2% and 87.9% of the cumulative variance of the N₂O fluxes at the undisturbed and disturbed marsh sites, respectively, and soil NO₃⁻-N contents and soil moisture were the predominant factors affecting spatial variability in N₂O emission (Fig. 4). The N₂O fluxes showed a significant negative correlation with soil moisture at the undisturbed or disturbed marsh sites. Almost no statistically significant correlations were obtained between N₂O fluxes and other environmental parameters at the undisturbed marsh sites. However, a strong positive correlation occurred between the fluxes and NO₃⁻-N contents at the disturbed sites, but the weak negative correlations between the fluxes and TOC, TN and NH₄⁺-N (Table 2), indicating that animal-derived NO₃⁻-N input significantly enhanced N₂O emissions from the SLM.

NEE and ER

NEE between dry, mesic and wet marsh sites showed a significant difference ($P < 0.05$), and the maximum mean CO₂ uptake occurred at the dry sites SD and WI ($-443.6 \pm 17.0 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ and $-284.7 \pm 90.1 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$, respectively) (Table 1). The marsh experienced a net gain of CO₂ in the summer or in the winter (Fig. 2). Overall net CO₂ emission occurred in the poultry active area ($29.1 \pm 33.2 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$), whereas waterbird activity area ($-140.0 \pm 41.9 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$), landfill ($-82.6 \pm 86.6 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$) and farmland ($-92.2 \pm 210.9 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$) showed weak CO₂ uptake compared to the non-disturbed marsh areas (Fig. 3). The marsh NEE had a strong positive correlation with soil moisture at both the undisturbed and disturbed sites, and soil moisture played a more important role in explaining the spatial variability in net CO₂ fluxes (Fig. 4 and Table 2).

The mean ER rate at the dry site ($1106.9 \pm 109.7 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$) was greatly higher than those at the mesic ($674.9 \pm 210.8 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$) and wet ($694.2 \pm 420.1 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$) sites (Fig. 5a and Table 1). Marsh ER showed a significant negative correlation with soil moisture ($P < 0.05$). However, the pattern of ER appeared to follow soil temperature with a strong, positive exponential correlation ($R^2 = 0.57$, $P < 0.05$) and the Q₁₀ value of 8.7 (Fig. 5b). Summertime photosynthesis (Pg) rates at the dry, mesic, paddy and wet sites averaged $1550.4 \pm 126.7 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$, $1020.4 \pm 202.8 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$, $1286.2 \pm 386.2 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ and $820.4 \pm 341.6 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$, respectively, and showed a large spatial variability between the types of sites (CV=8.2%, 19.9%, 30.0% and 41.6%, respectively). The Pg rates at the dry marsh

(1550.4 ± 126.7 mg CO₂ m⁻² h⁻¹) and paddy sites (1286.2 ± 386.2 mg CO₂ m⁻² h⁻¹) were significantly higher than those at the mesic (1020.9 ± 202.8 mg CO₂ m⁻² h⁻¹) and wet marsh sites (820.4 ± 341.6 mg CO₂ m⁻² h⁻¹) (Fig. 5a).

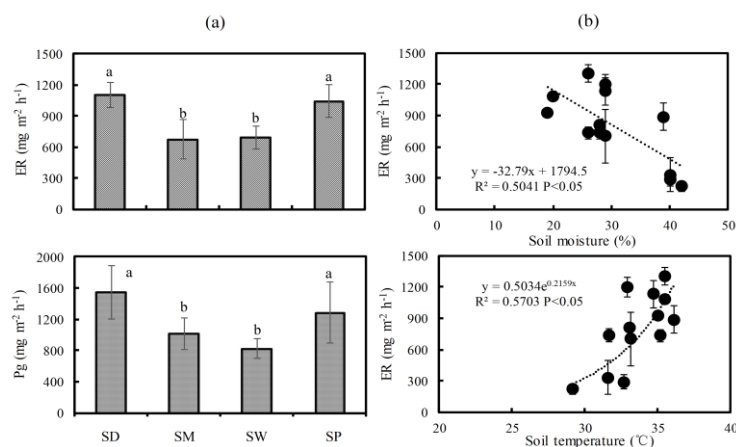


Figure 5. (a) The ecosystem respiration rates (ER) and gross photosynthesis (Pg) from the marsh transect sites during the summertime. The different lowercase letters (a, b) indicate statistically significant differences between means (Fisher's LSD, $P < 0.05$); (b) The relationship between ER and soil moisture, soil temperature at the marsh transect sites. If the overall regression is significant at $P < 0.05$, regression lines and r values are given

Correlation between GHG fluxes and global warming potential

The N₂O and CH₄ fluxes from different types of marsh sites showed a significant negative correlation, i.e. so-called “trade-off effect”. The significant positive relationships occurred between CH₄ flux and ER, NEE, whereas N₂O fluxes showed a significant negative correlation with NEE (Fig. 6). The average CH₄ and N₂O fluxes are $178.8 \mu\text{g m}^{-2} \text{h}^{-1}$ ($n=76$) and $15.2 \mu\text{g m}^{-2} \text{h}^{-1}$ ($n=76$), respectively, and their fluxes are equivalent to $4.5 \text{ mg CO}_2 \text{ m}^{-2} \text{h}^{-1}$ and $4.5 \text{ mg CO}_2 \text{ m}^{-2} \text{h}^{-1}$, respectively. However, net mean CO₂ flux from the marsh is $-212.6 \text{ mg m}^{-2} \text{h}^{-1}$. A GWP-negative process strongly dominates when N₂O, CH₄ and CO₂ fluxes from the marsh are combined in terms of their relative GWPs. Overall the SLM is still a large “carbon sink”.

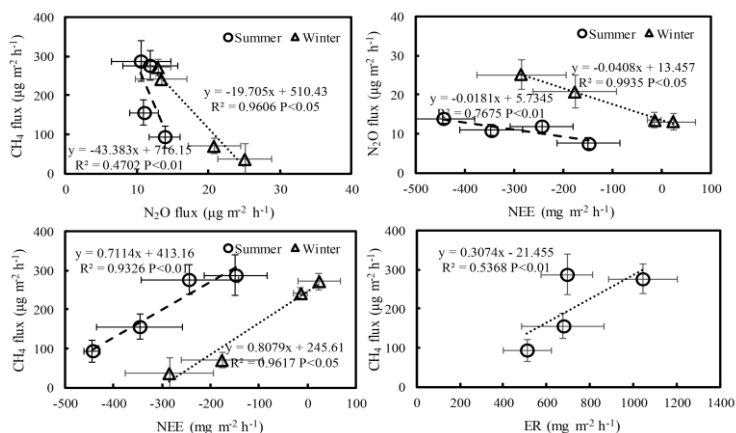


Figure 6. Correlations between CH₄ flux, N₂O flux, NEE and ER from different types of marsh sites

Discussion

Effects of soil moisture on spatial variations of GHG fluxes

Overall our statistical analyses showed soil temperatures and soil nutrient variables were not predominant factors affecting marsh CH₄ and CO₂ fluxes at the undisturbed sites (Table 2). Soil moisture (Mc) had a significant difference among the sites due to the drawdown of lake level and the differences in topography, slope and hydrology, which influenced the magnitudes and variations of GHG fluxes at spatial scale (Ding et al., 2002; Song et al., 2009). Mc showed a significant positive correlation with CH₄ fluxes and NEE, but a significant negative correlation with N₂O (Fig. 4 and Table 2). This indicated that marsh soil moisture regulated spatial variability in marsh GHG fluxes. Soil water regime is widely recognized as the fundamental factor in regulating small-scale variation in CH₄ emissions from the wetlands, given water regime can directly affect the anaerobic condition, which is necessary for CH₄ production (Christensen et al., 2003; Wang et al., 2008; Wei and Wang, 2017), whereas low soil moisture make O₂ diffuse into soil to oxidize CH₄ into CO₂ (Song and Liu, 2016; Bao et al., 2018a). Therefore the decrease in soil moisture inhibited marsh CH₄ production. N₂O is an intermediate product of soil nitrification and denitrification processes, and soil moisture might have an important effect on N₂O production through its effects on nitrification and denitrification processes (Granli and Bockman, 1994). In this study, N₂O fluxes showed a significant positive correlation with soil NO₃⁻-N contents, indicating that denitrification might be the main process responsible for marsh N₂O emissions. Furthermore increasing soil moisture could cause high anaerobic condition and low O₂ availability, and most of N₂O can be reduced to N₂ via denitrification, which might cause the decrease in N₂O emissions from the wetlands (Kato et al., 2011; Zhu et al., 2014b; Bao et al., 2018b). The lowering of water table could increase the volume of modestly aerated soil, thus increased the proportion of N₂O production in either of nitrification and denitrification (Kato et al., 2011; Chen et al., 2013). The incomplete denitrification releases N₂O, which might be favorably produced in marsh soil at well-drained, aerated marsh sites (Granli and Bockman, 1994; Scaroni et al., 2014). Therefore higher N₂O emissions occurred at the dry marsh sites SD, WD and WI in the SLM.

On the other hand, the decrease in soil moisture might stimulate carbon sequestration in the marsh (Fig. 2). Stronger plant photosynthesis occurred at the site SD than SM and SW (Fig. 5a), which might be due to higher vegetation coverage at the dry upland marsh (Lu and Liu, 2011; Yang et al., 2015). Soil moisture directly affected the distribution of soil microbial communities and marsh vegetation, thus influenced ecosystem respiration (Llstedt et al., 2000). Previous studies indicated that the Pg rates were the highest in dry tundra marsh with smaller differences in net CO₂ exchange whereas the ER rates were limited, therefore the dry marsh showed much higher net CO₂ uptake (Dagg and Lafleur, 2011). In the SLM, the decrease in soil moisture stimulated ER rates instead of limiting ER (Fig. 5b). The increase in net CO₂ uptake at the dry marsh sites showed that plant photosynthesis might surpass ER with the drawdown of lake water level. Similarly, plant photosynthesis, rather than the mineralization of organic matter, played a more important role in net CO₂ flux at the dry marsh site (Del Giorgio et al., 1999; Huttunen et al., 2003). Therefore plant photosynthesis might dominate CO₂ exchange across air-soil interface in the SLM. In addition, a strong, positive exponential correlation between ER and soil temperature

showed a positive feedback of ER to current warming climate. Climate warming might decrease CO₂ sink through the increase in ER (Davidson and Janssens, 2006; Zhu et al., 2014a). The Q₁₀ value of 8.7 in the SLM was much higher than the range (1.3-5.6) for different biomes of the world (Davidson and Janssens, 2006), indicating a greater temperature sensitivity to ER.

Effects of animal activity and land use on GHG fluxes

Overall the mean CH₄ fluxes from waterbird and poultry active areas showed no statistical significant differences ($p > 0.05$) from the undisturbed sites (*Fig. 3*). The fluxes from waterbird ($148.3 \pm 51.5 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$) and poultry ($64.4 \pm 44.3 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$) active areas were lower than those from dairy cow feeding yards ($358.3 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$), dairy cow collecting yards ($429.2 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$), and pig loading areas ($158.3 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$) (Misselbrook et al., 2001), which might be related with low soil moisture, especially in poultry active area ($\text{Mc} < 30\%$, *Table S2*). Lower soil moisture might greatly increase marsh CH₄ consumption (Gulledge and Schimel, 1998). However, net CO₂ emission occurred in the poultry active area ($29.1 \pm 33.2 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$), whereas waterbird activity area showed weak CO₂ uptake compared to non-disturbed marsh areas, indicating that poultry activity could decrease marsh CO₂ sink, even convert a marsh site from CO₂ sink into a net source. Similar results also occurred in the tundra marsh disturbed by marine animal activities (Zhu et al., 2014a).

The N₂O fluxes from poultry (mean $49.5 \pm 11.9 \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$) and waterbird (mean $14.1 \pm 3.9 \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$) active areas were comparable to or even greater than those from the farm yards used by livestock ($11.8\text{--}27.5 \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$) (Misselbrook et al., 2001), and from some temperate extensive pastures or rangelands ($0\text{--}26.6 \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$) (Phillips et al., 2007), and those from heavily fertilized agricultural systems and tropical forests ($23.6\text{--}83.3 \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$), which are the two largest N₂O sources worldwide (Perez et al., 2001). Similar results also occurred in tundra marsh sites disturbed by seabird activities ($13.3\text{--}57.4 \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$) (Zhu et al., 2012, 2013). The high N₂O emissions from poultry or waterbird active areas might be modulated by soil physical and chemical processes associated with animal activities: continuous N input from animal excreta and animal tramp (Zhu et al., 2012). Significantly elevated NO₃[−]-N concentrations occurred in animal-disturbed marsh soils as compared with undisturbed soils (*Table S2*). A strong positive correlation between N₂O fluxes and soil NO₃[−]-N contents confirmed that animal-derived NO₃[−]-N input had an important effect on spatial variability in N₂O fluxes (*Fig. 4* and *Table 2*). Therefore, animal activity and the deposition of their excreta significantly enhanced N₂O emissions in the SLM.

During the conversion of the marsh into paddy fields, human tillage and the rice plants with well-developed aerenchyma might lead to the increase in CH₄ emission (Cai et al., 1997). The plants with well-developed aerenchyma are important in determining net CH₄ fluxes from some wetlands (Bass et al., 2014; Bao et al., 2018b). However, net CH₄, N₂O and CO₂ fluxes from agricultural field site FD were comparable to the undisturbed sites (*Fig. 3*), indicating that the conversion of the marsh into agricultural fields did not significantly change GHG emissions. Landfill gas is mainly composed of CH₄ and CO₂, which is generated during decomposition of the solid waste (Sharma et al., 2011). The landfill did not change CH₄ and N₂O fluxes, and slightly decreased CO₂ uptake compared to non-disturbed marsh areas (*Fig. 3*). GHG fluxes from the landfill were lower than those reported from sanitary landfills (Bogner et al., 1995; Rinne et al., 2005; Kormi et al., 2017). Generally landfill GHG emission estimates are highly

uncertain because factors influencing gas emission and mitigation are site specific (Boeckx et al., 1996). Landfill GHG emissions can range over six orders of magnitudes (Lando et al., 2017). The ambient and soil moisture had an important effect on GHG production from the landfill (Rinne et al., 2005). During the study period, very high soil moisture (68%-73%, *Table S2*) might stimulate CH₄ and CO₂ emissions, but decrease N₂O production from the landfill (*Fig. 3*).

In summary, correlation analyses revealed that GHG fluxes from the disturbed marsh sites were involved in more complicated factors than from undisturbed sites (*Table 2*). Significant positive correlations ($p < 0.05$) between net CH₄ and CO₂ fluxes and soil moisture, soil nutrients indicated that their fluxes might be modulated by soil physical and chemical processes associated with animal activities (Zhu et al., 2012, 2013). Negative correlations were generally observed between N₂O emissions and soil moisture, nutrient variables TOC, TC, TN and NH₄⁺ at the disturbed sites and all the combined sites (*Fig. 4* and *Table 2*), which was different from some previous studies (Chen et al., 2011; Sun et al., 2014). One possible reason was related to the interaction of vegetation and microorganism (nitrifiers and denitrifiers) during N₂O production (Sun et al., 2014). N₂O production might be partly inhibited as the available N was significantly competed by both vegetations and microorganisms in *Artemisia lavandulaefolia* DC and *Artemisia scoparia* waldst community (Lu and Liu, 2011; Yang et al., 2015), which partly contributed to the difference in N₂O emissions at spatial scale. Overall the PCA analysis showed that soil moisture was the predominant factor affecting spatial variability in marsh GHGs fluxes from the undisturbed and disturbed sites in the SLM (*Fig. 4*). This preliminary study only provided short summertime or wintertime GHG fluxes from agricultural field or landfill in the SLM, and effects of animal activities and land use on marsh GHG emissions need to be further confirmed in the future.

Implication of marsh GHG evaluation

The SLM, as a typical natural freshwater wetland in the Middle-Lower Yangtze Plain, is involved in effects of complicated factors including environmental variables, human activities and waterbird colonization (Lu and Liu, 2011; Yang et al., 2015; Zhang et al., 2015). The coefficients of variance (CV) of CH₄, N₂O and CO₂ fluxes were relatively small (<100%) within the same type of sites. However, their fluxes showed significant differences among the sites, indicating that to assess the regional GHG budget precisely, measurements should be designed at fine scales and environmental heterogeneity should be considered in the marsh. In addition, the study area has a temperate monsoon climate with an evident distinction in precipitation between rainy and dry seasons. This could lead to soil moisture changes, dry-wet alternation and wetland extent dynamics, and cause the “trade-off effect” of N₂O and CH₄ fluxes and carbon sequestration in the marsh (Cai et al., 1997; Ding and Cai, 2007). Effects of soil moisture changes on “trade-off effect” of N₂O and CH₄ fluxes need to be used for accurate evaluation of net GWP in the SLM. In summary, there still remains a large uncertainty regarding the evaluation of GHG budgets in the SLM. The GHG budget evaluation for the SLM and other similar MLYP wetlands should be involved in soil water regimes, waterbird colonization and human-induced influences.

Conclusions

We revealed effects of soil moisture, animal activities and land use on GHG fluxes from the SLM based upon *in situ* flux measurements. Overall CH₄ emissions from the dry marsh sites were much lower than those from wet or mesic sites, vice versa for N₂O emissions and NEE. Soil moisture was the predominant factor controlling the spatial variability in marsh CH₄ and N₂O fluxes and NEE at undisturbed sites. Correlation analyses revealed that GHG fluxes from the disturbed marsh sites were involved in more complicated factors than from undisturbed sites. The mean CH₄ fluxes from waterbird and poultry active areas showed no statistically significant differences ($p > 0.05$) from the undisturbed sites, but animal activities increased N₂O emissions, decreased marsh CO₂ sink, even convert into a net CO₂ source. The conversion of the marsh into agricultural field and landfill did not significantly change GHG emissions compared to non-disturbed marsh areas. To assess the regional GHG budget precisely, measurements should be designed at fine scales and environmental heterogeneity should be considered in the marsh. This study only provided short summertime and wintertime GHG fluxes, and long-term and high-frequency observations are needed to accurately quantify GHG budgets in the SLM. Continued investigations into the dynamics of GHG fluxes as well as mechanisms of GHG production in natural wetland, with an emphasis on temporal variation are essential in the future.

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APPENDIX

Electronic Appendix: The detailed information about investigation sites, experimental design and soil physiochemical properties at all the stations were summarized in the Appendix.