

## APPENDIX

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

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### S1. Study Area

The Shengjin Lake Wetland National Nature Reserve (116° 55' E-117°15' E, 30° 15' N-30° 30' N) is located at the junction of Dongzhi County and Guichi district in Anhui Province and in the Middle-Lower Yangtze Plain near the Yangtze River. The Shengjin Lake is an important flood storage/discharge reservoir and a natural fishing ground, and it is surrounded by diverse terrains with curving lakeshore lines. Topographically it can be divided into three parts: the upper lake, the middle lake and the lower lake. At present, there are 13 towns and 46 villages around the lake with total population of about 80,000 (Jiang et al., 2007). Most of the villagers depend on the cultivation of cotton and rice on the plain around the lake. Grazing livestock in the lake marsh and fishery resource from the Shengjin Lake are also essential food sources for the local residents (Fox et al., 2013).

The Shengjin Lake Marsh (SLM) has similar hydrological and ecological features to other Chinese wetlands in mid-latitude area. The marsh around the lake has diverse vegetation types including *Artemisia lavandulaefolia* DC, *Artemisia scoparia* Waldst and *pinus massoniana* (Lu and Liu, 2011; Bernal and Mitsch, 2012; Yang et al. 2015). Human activities including the land use conversion and aquaculture, and natural disturbances have intensively affected the local environment (Yang et al., 2015). This marsh 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). Migrant birds play an important role in the nutrient cycling in the ecosystems, and their activities strongly influence the physical and chemical properties for the marsh soils, which might lead to be generally high in soil organic carbon, total nitrogen and total phosphorus levels (Lu and Liu, 2011; Zhu et al., 2014a, b). Overall the marsh has been experiencing human disturbance from the conversion of land use, and natural disturbance from waterbirds, drought and flooding (Lu and Liu, 2011).

## S2. Investigation Sites and Experimental Design

(i) The upland marsh area: During the rain season in July 2013, the lake water level rose up to the highest, and the marsh was formed along the lakeshore. The total 14 stations (sj1-1 to sj1-14) for GHG flux observations were set up from the dry to waterlogged marsh areas along the lakeshore transect.

(ii) The lowland marsh area: A large flooded land area was exposed with the decrease in the lake water level during the dry season in December 2013. We selected a long transect from the upland to the lowland, and then to a small island along the lakeshore. The middle lowland was exposed only during the dry season in winter, and the upland and the island were both exposed areas throughout the year.

(iii) The marsh sites disturbed by animal activities and land use: Four disturbed areas including waterbird active area (BA), poultry active area (PA), landfill (LF) and farmland (FD), were identified as the observation areas for GHG fluxes. The waterbird active area was located near the transect. A great number of migrant birds flew to the lake marsh to live through the winter, and their guano could be seen everywhere in the exposed marsh. The poultry active area was located in the northwest of the marsh. A lot of geese and ducks often went into the area, and their fertile excreta deposited into the marsh soils. The ground surface in the poultry and waterbird active areas had sparse vegetation due to the tramp of the animals and the input of high nutrients from their excreta. The landfill was a bog with the soft peat and a large amount of garbage. The marsh exposed in summer was transformed into the farmland where winter wheat grew, resulting in soft soil texture and low soil moisture. Two repetitions were made at each station. The soil physiochemical properties at all the microsites were summarized in *Table S1* and *Table S2*.

*Table S1. Soil physical and chemical properties at the upland marsh area.*

Marsh sites	pH	TOC (%)	TN (%)	TC (%)	TS (%)	NH <sub>4</sub> <sup>+</sup> -N (µg g <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> N (µg g <sup>-1</sup> )	Mc (%)
Stations at wet site (SW)								
sj1-1	5.9	0.79±0.15	0.13±0.01	0.98±0.19	0.11±0.01	9.2±3.6	203.7±268.0	40
sj1-2	5.5	1.06±0.12	0.18±0.02	1.10±0.09	0.09±0.03	3.6±0.6	429.4±291.6	42
sj1-3	5.5	1.11±0.04	0.19±0.02	1.16±0.09	0.04±0.01	5.0±0.7	905.4±234.5	40
sj1-4	5.2	1.25±0.05	0.20±0.00	1.29±0.01	0.03±0.00	6.9±3.0	990.1±147.1	40
Mean	5.5±0.1a	1.05±0.10a	0.18±0.02a	1.13±0.06a	0.07±0.02a	6.2±1.2a	632.2±188.7ab	41±1a
Stations at mesic site (SM)								
sj1-5	6.1	1.28±0.03	0.20±0.01	1.36±0.03	0.03±0.00	9.6±0.7	727.0±404.4	28
sj1-6	5.7	1.25±0.13	0.20±0.01	1.32±0.12	0.02±0.00	1.8±2.6	1120.7±172.4	28
sj1-7	5.9	0.89±0.07	0.16±0.01	0.99±0.04	0.02±0.00	5.9±2.1	669.3±547.6	29
sj1-8	5.7	0.88±0.06	0.17±0.01	1.02±0.02	0.01±0.00	4.7±1.8	451.9±165.6	26
sj1-9	5.7	0.87±0.01	0.17±0.00	1.08±0.04	0.01±0.00	1.2±0.7	1736.7±1458.4	29

Mean	5.8±0.1a	1.03±0.09a	0.18±0.01a	1.15±0.08a	0.02±0.00b	4.6±1.5a	941.1±226.3b	28±1b
Stations at dry site (SD)								
sj1-10	5.8	0.96±0.05	0.16±0.00	1.06±0.05	0.01±0.00	6.3±0.9	839.3±450.4	26
sj1-11	5.8	1.47±0.19	0.21±0.01	1.64±0.21	0.02±0.00	5.2±2.1	735.1±140.4	20
sj1-12	5.6	1.32±0.07	0.20±0.01	1.45±0.03	0.01±0.00	4.7±1.7	968.8±440.3	19
Mean	5.7±0.1a	1.25±0.15a	0.19±0.02a	1.38±0.17a	0.01±0.00b	5.4±0.5a	847.7±67.6b	22±2c
Stations at paddy site (SP)								
sj1-13	5.9	1.41±0.06	0.18±0.01	1.56±0.06	0.01±0.00	21.5±3.1	85.2±60.1	39
sj1-14	5.7	1.33±0.09	0.18±0.00	1.41±0.07	0.01±0.00	9.0±2.7	210.9±64.1	29
Mean	5.8±0.1a	1.37±0.04a	0.18±0.00a	1.49±0.08a	0.01±0.00b	15.3±6.3b	148.1±62.9a	34±5d

Note: TOC, TN, TC, TS, Mc indicate total organic carbon, total nitrogen, total carbon, total sulfur and soil moisture respectively. The measured soil physical and chemical properties at the upland marsh transect sites with the same suffix letter (a, b, c or d) are not significantly different from one another (LSD,  $P < 0.05$ ).

**Table S2.** Soil physical and chemical properties at the lowland marsh area and the marsh sites disturbed by animal activities and land use.

Marsh sites	TOC (%)	TN (%)	TC (%)	TS (%)	NH <sub>4</sub> <sup>+</sup> -N (µg g <sup>-1</sup> )	NO <sub>3</sub> -N (µg g <sup>-1</sup> )	Mc (%)
Stations at the lowland marsh area							
Stations at dry site (WD)							
sj2-1	0.64±0.25	0.13±0.03	1.05±0.41	0.24±0.05	6.0±1.1	23.0±171.5	23
sj2-2	1.82±0.91	0.29±0.08	2.42±1.03	0.17±0.03	7.5±14.7	281.1±231.6	42
sj2-3	2.14±0.10	0.35±0.01	2.75±0.09	0.13±0.01	22.0±9.2	47.8±73.0	46
sj2-4	2.57±1.30	0.42±0.15	3.57±1.73	0.13±0.03	44.5±41.9	158.9±277.7	42
Mean	1.79±0.41ac	0.30±0.06ac	2.45±0.52ad	0.17±0.03d	20.0±8.9a	127.7±59.1a	38±5a
Stations at mesic site (WM)							
sj2-5	3.54±0.21	0.48±0.03	4.46±0.35	0.12±0.01	59.4±38.1	355.3±427.2	51
sj2-6	3.16±1.25	0.50±0.11	4.13±1.23	0.12±0.01	116.6±13.4	97.3±27.6	51
sj2-7	2.71±0.58	0.50±0.05	4.02±0.31	0.11±0.00	25.4±8.9	87.7±65.3	54
Mean	3.14±0.24a	0.49±0.01a	4.20±0.13b	0.12±0.00a	67.1±26.6bc	180.1±87.6a	52±1b
Stations at wet site (WW)							
sj2-8	2.93±0.40	0.46±0.01	4.27±0.09	0.09±0.00	49.3±33.0	108.0±45.0	55
sj2-9	3.21±0.10	0.49±0.02	4.49±0.27	0.08±0.01	65.9±1.6	154.0±39.9	59
sj2-10	1.04±0.24	0.23±0.01	1.69±0.10	0.05±0.00	5.8±1.4	712.5±310.6	55
Mean	2.39±0.68a	0.39±0.08ac	3.48±0.90ab	0.07±0.01ab	40.3±17.9ab	324.8±194.3a	56±1bd
Stations at dry island site (WI)							
sj2-11	3.51±0.15	0.53±0.01	4.66±0.06	0.09±0.01	22.3±15.2	27.8±25.3	45
sj2-12	1.23±0.03	0.24±0.02	1.70±0.05	0.04±0.00	9.6±2.3	27.2±34.4	20
sj2-13	1.96±0.44	0.28±0.04	2.44±0.54	0.05±0.00	22.2±4.1	27.3±34.6	20
sj2-14	1.49±0.38	0.25±0.03	2.07±0.48	0.04±0.00	19.5±7.0	7.8±15.4	17

Mean	2.05±0.51a	0.33±0.07ac	2.72±0.66abd	0.06±0.01b	18.4±3.0a	22.5±4.9a	26±7c
Stations at the marsh sites disturbed by animal activities and land use							
Stations at the landfill site (LF)							
sj2-15	9.10±1.31	1.10±0.10	10.62±1.10	0.18±0.2	123.3±13.4	784.4±685.9	73
sj2-16	11.16±0.02	1.46±0.15	13.37±0.06	0.19±0.04	53.8±36.8	1502.4±1282.7	68
Mean	10.13±1.03b	1.28±0.18b	12.00±1.38c	0.19±0.01d	88.6±34.8bc	1143.4±359.0a	71±3d
Stations at the poultry active site (PA)							
sj2-17	0.61±0.15	0.17±0.08	1.14±0.33	0.01±0.01	4.7±1.9	2474.0±127.9	19
sj2-18	0.58±0.09	0.16±0.02	1.10±0.23	0.02±0.01	3.4±2.7	2727.0±703.4	20
sj2-19	0.56±0.02	0.18±0.02	1.03±0.03	0.03±0.01	1.2±0.1	2273.3±246.2	20
sj2-20	1.22±0.46	0.33±0.06	2.00±0.42	0.07±0.00	2.8±3.9	4939.6±2381.2	26
Mean	0.74±0.16c	0.21±0.04c	1.32±0.23d	0.03±0.01bc	3.0±0.7a	3103.5±619.0b	21±2c
Stations at water bird active site (BA)							
sj2-21	2.04±0.05	0.41±0.01	2.76±0.05	0.09±0.00	36.2±3.0	1009.7±346.0	46
sj2-22	2.42±0.04	0.45±0.01	3.21±0.01	0.10±0.01	49.0±26.7	767.5±365.9	51
Mean	2.23±0.19ac	0.43±0.02ac	2.99±0.23abd	0.10±0.01ab	42.6±6.4abc	888.6±121.1a	49±3ab
Stations at farmland site (FD)							
sj2-23	1.92±0.24	0.39±0.03	2.84±0.19	0.09±0.01	107.3±4.0	628.1±185.5	43
sj2-24	1.23±0.12	0.27±0.01	1.81±0.19	0.06±0.00	72.2±1.0	1201.4±76.2	42
Mean	1.58±0.35ac	0.33±0.06ac	2.33±0.52abd	0.08±0.02ab	89.8±17.6c	914.8±286.7a	43±1ab

Note: TOC, TN, TC, TS, Mc indicate total organic carbon, total nitrogen, total carbon, total sulfur and soil moisture respectively. The measured soil physical and chemical properties at the upland marsh transect sites with the same suffix letter (a, b, c or d) are not significantly different from one another (LSD,  $P < 0.05$ ).

### S3. Determination of GHG Concentrations and Flux Calculation

The CH<sub>4</sub> concentration was tested by gas chromatography (Shimadzu GC-12A, Japan) with flame ionization detector (FID), and the column and detector temperatures were 80 and 200 °C, respectively. The N<sub>2</sub>, H<sub>2</sub> and air flow rates were 40 ml min<sup>-1</sup>, 35 ml min<sup>-1</sup> and 350 ml min<sup>-1</sup>, respectively (Zhu et al., 2009a, b). The N<sub>2</sub>O concentration was determined by the gas chromatography (GC-HP5890, USA) with the <sup>63</sup>Ni electron capture detector (ECD). The Ar-CH<sub>4</sub> (95:5) was used as the carrier gas, and the flow rate was 30 ml min<sup>-1</sup>; the column and detector temperatures were 85 and 330 °C, respectively. The back flush time was 2.80 min and the volume of the sample injector is 1.7 ml (Chen et al., 2013). The CO<sub>2</sub> concentration was determined equipped with the gas chromatography (GC) with thermal conductivity detector (TCD) (Shimadzu GC-14B, Japan) and 80/100 mesh Chromosorb 102 column. The oven, injector and detector temperatures were 60, 100 and 60 °C, respectively. The carrier gas (H<sub>2</sub>) had a flow rate of 80 ml min<sup>-1</sup>. The variance coefficient for standard samples was within 0.2-0.5% in 24 h (Zhu et al., 2009a, b).

The GHG fluxes were calculated using a linear least squares fit to the four points in the time series (0, 10, 20, and 30 min during the time of chamber closure) 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 the 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 GHG fluxes were included regardless of their  $R^2$  value. For the gas fluxes, positive values indicate net emission to the atmosphere, and negative values indicate net uptake from the atmosphere.

## REFERENCES

- [1] Bernal, B., Mitsch, W.J. (2012): Comparing carbon sequestration in temperate freshwater wetland communities. – *Glob. Chang. Biol.* 18:1636-1647.
- [2] Chen, Q.Q., Zhu, R.B., Xu, H. (2013): Spatial and temporal variations in CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O concentration in Ny-alesund, Svalbard. – *Adv. Polar Sci.* 25:150-160.
- [3] Fox, A.D., Hearn, R.D., Cao, L., Cong, P.H., Wang, X., Zhang, Y., Dou, S.T., Shao, Z.F., Barter, M., Rees, E.C. (2013): Preliminary observations of diurnal feeding patterns of Swan Geese *Anser cygnoides* using two different habitats at Shengjin Lake, Anhui Province, China. – *Wildfowl* 58:20-30.
- [4] Jiang, H.X., Xu, W.B., Qian, F.W., Chu, G.Z. (2007): Impact of habitat evolution and human disturbance on wintering water birds in Shengjin Lake of Anhui Province, China. – *Chinese J. Appl. Ecol.* 18:1832-1836.
- [5] Lu, Z.M., Liu, L. (2011): Investigation and analysis of Present Wetland Environmental Situation of Shengjin Lake. – *J. Chizhou Coll.* 6:80-83 (in Chinese).
- [6] Yang, S.W., Dong, B., Liu, L.P., Sun, L., Sheng, S.W., Wang, Q., Peng, W.J., Wang, X., Zhang, Z.F., Zhao, J. (2015): Research on Vegetation Coverage Change in Sheng Jin Lake Wetland of Anhui Province. – *Wetlands* 35:677-682.
- [7] Zhang, Y., Jia, Q., Prins, H.H.T. (2015): Effect of conservation efforts and ecological variables on waterbird population sizes in wetlands of the Yangtze River. – *Sci. Rep.* 5:17136.
- [8] Zhu, R.B., Bao, T., Wang, Q., Xu, H., Liu, Y.S. (2014a): Summertime CO<sub>2</sub> fluxes and ecosystem respiration from marine animal colony tundra in maritime Antarctica. – *Atmos. Environ.* 98:190-201.
- [9] Zhu, R.B., Liu, Y.S., Ma, E.D., Sun, J.J., Xu, H., Sun, L.G. (2009a): Greenhouse gas emissions from penguin guanos and ornithogenic soils in coastal Antarctica: effects of freezing-thawing cycles. – *Atmos. Environ.* 43: 2336-2347.
- [10] Zhu, R.B., Liu, Y.S., Ma, E.D., Sun, J.J., Xu, H., Sun, L.G. (2009b): Nutrient compositions and potential greenhouse gas production in penguin guano, ornithogenic soils and seal colony soils in coastal Antarctica. – *Antarct. Sci.* 21:427-438.

[11] Zhu, R.B., Ma, D.W., Xu, H. (2014b): Summertime N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub> exchanges from a tundra marsh and an upland tundra in maritime Antarctica. – *Atmos. Environ.* 83:269-281.