Responses of N_2O flux to water level fluctuation and other environmental factors at littoral zone of Miyun Reservoir: a comparison with CH_4 fluxes

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Abstract

There have been only a few studies that allow us to estimate the contribution of reservoirs to greenhouse gas budgets. In particular, information is limited for understanding the spatiotemporal variation of N_2O flux and the underlying mechanisms in the littoral zone where complex biochemical processes are induced by water level fluctuations. A study was carried out at five different water levels (deep water, shallow water, seasonally flooded, control for seasonally flooded, and non-flooded) all within the littoral zone of a temperate reservoir using the static chamber technique. The 'control for seasonal flooded' had similar vegetation to the 'seasonally flooded' but was not actually flooded as it was on a higher piece of land. Seasonal, diurnal and spatial variations of N_2O flux and environmental factors were monitored throughout the growing season including a flood event during summer rains. The N_2O flux ranged from –136.6 to 381.8 μg m^{-2} h^{-1} averaging 6.8 μg m^{-2} h^{-1}. Seasonal and spatial variation was significant but diurnal variation was not. Non-flooded dry land emitted more N_2O than flooded land, no matter whether it was permanently or seasonally flooded. Piecewise correlation was found between N_2O flux, air temperature and soil nitrate. Positive correlation was shown between N_2O flux and dissolved oxygen in water. Besides deep water area, contrasting sampling between natural land and farmland (maize) was carried out

showing significant higher emission in farmland. In order to know the contrasting characteristics of N_2O and CH_4 fluxes in the littoral zone of the reservoir, results were compared with a previously published study of CH_4 emissions, carried out simultaneously at the same site as those in the present study. Completely different patterns between the two gases are demonstrated. In conclusion, the littoral zone is a hot-spot for N_2O in the summer, especially when the shores of the lake are used for farming of maize. But in terms of the overall greenhouse gas budget, the fluxes of N_2O are not as important as those of CH_4.

1 Introduction

Currently the greenhouse gas emissions from reservoirs are attracting the attention of researchers because these water bodies are increasing rapidly in number and area, growing with the continuing demand for water and hydropower. In rapidly developing countries like China, India and Brazil this growth is likely to continue for many years (Yang and Lu, 2014; Kumar et al., 2011). It is speculated that the construction of impoundments causes sediment accumulation and vegetation change, and when agricultural lands are inundated during creation of reservoirs, and for many years afterwards, there may be a strong enhancement of greenhouse gas emissions (Tranvik et al., 2009). It is noteworthy that this speculation is usually based on the expectation of an altered carbon cycle whilst data on aspects of the nitrogen cycle are lacking (L. Yang et al., 2014).

The pelagic zone of reservoirs have more often been studied (Beaulieu et al., 2014; Guérin et al., 2008; Huttunen et al., 2002; X. L. Liu et al., 2011) but there are limited studies in the littoral zone which may be a hotspot of N_2O emissions (Wang et al., 2006). In the few cases where it has been studied, N_2O emissions of the littoral zone in natural lakes have been observed to be higher than the pelagic zone even though the area differences had been taken into account (Huttunen et al., 2003).

Because of the strong gradients in water level and water level fluctuations, compared to the more or less stable pelagic zone and some other ecosystems (e.g. grassland and farmland), the environment of the littoral zone is more diverse and dynamic in terms of soil moisture, plant taxa and soil nutrients across scales of both space and time (Peng et al., 2011; Ahn et al., 2014; Trost et al., 2013). Those factors would in turn influence N_2O production (Lu and Xu, 2014). Limited previous studies on N_2O emissions of the littoral zone suggested significant spatio-temporal variations. But most of the studies just focus on a single water level (with different

communities sometimes) which might miss the spatial variations between different water levels (Chen et al., 2011b; Y. Liu et al., 2011). Temporally, reports always showed seasonal variation but not diurnal variation (Chen et al., 2010; Huttunen et al., 2003). To match the diverse and dynamic environment of the littoral zone, we combined five water levels on a transect from water to dry land, three plant communities for each water level including both natural and cropped land, six times during the year and seven times of day. The improved sampling both in space and time was expected to provide more representative data on N_2O emission of the littoral zone, and to provide further insights into the nature of the underlying processes.

To be more specific, the objectives of this present study included (i) capturing the spatial and temporal variation of the N_2O flux at the littoral zone of the Miyun Reservoir; (ii) finding the relationship between the observed flux and environmental factors; and (iii) evaluating the relative importance of N_2O and CH_4 fluxes by comparing with our earlier report of the CH_4 fluxes made simultaneously from the same site (M. Yang et al., 2014). The over-arching hypothesis in this work is: the littoral zone is a hot-spot of N_2O emissions that is influenced by seasonal changes in the water level.

2 Methods

2.1 Study area

The research was carried out at Miyun Reservoir (40°29′N, 116°50′E), which is located in the northern mountainous area of Beijing, China. It was built in 1960 with a maximum water area of 188 km^2. Its catchment is characterized by warm temperate semi-humid monsoonal climate with an annual average air temperature of 10.5°C, maximum air temperature of 38°C, and a minimum of −18°C. The reservoir is normally covered by ice from the middle of November to the end of March. The growing season is from April to November. The annual average precipitation is close to 600 mm, of which 80% is concentrated from July to August (Gao, 1989). Over 93% of the soils around the reservoir are classed as cinnamon soils (*korichnezems*) with typical soil pH from 7.0 to 8.2 (Anonymous, 2008). Alongside the reservoir, higher land (sometimes just slightly higher) is always used by local people for growing maize. This opportunistic agriculture is typically from May to September. Nitrogenous fertilizer is applied during sowing, and sometimes with further application in the

middle of the growing season. This reservoir is mainly used as the domestic water supply for Beijing. The water quality is controlled to level II according to Environmental Quality Standards for Surface Water of People's Republic of China GB3838-2002 (levels are rated on a scale I to V, where level I is the cleanest, available at: http://kjs.mep.gov.cn/hjbhbz/index.htm). The annual change in the water level is 1–5 m, reflecting the balance between rainfall, evaporation and usage. The area between the highest and lowest water level from 1984 to 2005 was 84 km^2 (Cao et al., 2008). In the summer of 2012, when the work was carried out, unusual and continuous heavy rain in July caused a sudden water level increase of 0.8 m in 15 days, and part of the littoral vegetation was inundated. This provided us with a seasonal flooded area which made possible an exploration of the effects of summer flooding on greenhouse gas emissions.

We divided the littoral zone into five areas based on water level (Fig. 1). Sites were selected ranging from locations in open water to the dry area on higher ground, to provide five contrasting environments: (i) deep water area (DW); (ii) shallow water area (SW); (iii) seasonal (August and September) flooded area (SF); (iv) 'seasonally flooded control' (SFC) area, which was 500 m away from SF, had the same plant species as SF, but escaped the flood in August and September because of its slightly (about 1 m) higher elevation; and (v) an area which is seldom flooded (the last flooding was several years ago) which hereafter we call the non-flooded area (NF). Three typical plant communities in each water level were selected. At SW, SF, SFC and NF, land cropped with maize (*zea mays*) was included as it is a typical practice, and allows some assessment of the impact of farming. Maize land in SW and SF was abandoned by the local farmer after our first sampling campaign because of flooding. So these lands were colonised by wild plants since the second campaign. Dominant species of each month are shown in Table 1. Details of climate, biomass and soil/sediment parameters are shown in Fig. 2 and Fig. 3.

2.2 N_2O flux measurements

Nitrous oxide flux was measured in November 2011, then May, July, August, September and October 2012. The experiment at site SFC was carried out just after the flooding and during the time when the water level dropped from August to October 2012. In order to reduce uncertainty in the average daily flux, a sampling protocol designed to capture any diurnal variation was performed at three-hourly intervals (local time: 6, 9, 12, 15, 18, 21 and 24 h). Each plot had four replicate chambers located within three meters from each other. To

eliminate disturbance to the soil/sediment during sampling, wooden access platforms were built.

The static opaque chamber technique was used to determine the N_2O flux. The chambers were made of stainless steel (volume: 125 litres; surface area: 0.25 m^2) and coated with polyethylene foam to minimize any warming effect inside the chamber. An extension chamber (volume: 200 litres; surface area: 0.25 m^2) was added if plants were tall. Two fans were built into the chamber for air mixing. Four gas samples (200 ml each) were taken using 100-ml polypropylene syringes at 15-min intervals over a 45-min period after enclosure, and stored in 500-ml plastic and aluminum membrane gas sampling bags (Guangming Research and Design Institute of Chemical Industry, China). The concentration of N_2O was analyzed within one week by gas chromatography (7890A, Agilent, USA) equipped with a micro-electron capture detector (μ-ECD). Gases were separated with a column (3 m, 3.2 mm) packed with Porpak Q (80/100 mesh). The temperatures of the oven, injector, and detector were 70°C, 20°C, and 330°C, respectively. The flow rate of the carrier gas (N_2) was 25 ml min^{-1}. Standard N_2O gas (310 ppb in air, China National Research Center for Certified Reference Materials, China) was used for precision verification for N_2O concentrations. The coefficient of variation was below 1.5%. The flux of N_2O was calculated following Chen et al. (2011b):

$$F = \frac{M}{V_0} \times \frac{P}{P_0} \times \frac{T_0}{T} \times \frac{\mathrm{d}C_t}{\mathrm{d}t} \times H \quad (1)$$

where F is the flux of N_2O (mg m^{-2} h^{-1}); M is the molar mass of N_2O (g mol^{-1}); P (kPa) is the atmospheric pressure of the sampling site; T (K) is the absolute temperature of the sampling time; V_0 (22.4 L), P_0 (101.325 kPa) and T_0 (273.15 K) is the molar volume, atmosphere pressure and absolute temperature, respectively, under standard conditions; dC_t/dt (ppm h^{-1}) is the rate of concentration change; and H (m) is the chamber height over the water or soil surface.

Chambers were reset into new positions near the old positions each sampling month. All positions at each site were within an area of 20 m^2, but not so close to each other to cause artifacts in the data through (for example) changes in the local hydrology.

2.3 Environmental factors

Weekly precipitation was accessed through the China Meteorological Data Sharing Service System (http://www.escience.gov.cn/metdata/page/index.html). Average wind speed was recorded during the sampling period with a hand-held vane anemometer (4101, Testo, Germany), taking an average over the 45 minute period during which gas was sampled. Air temperature was measured by a digital thermometer (JM624, Jinming, China) at the start and end of each gas sampling at every plot. Dissolved Oxygen (DO) in water was measured during the gas sampling by a handheld multi-parameter meter (Professional Plus, YSI, USA). The aboveground biomass of every replicate in the chamber was weighed after drying at 80°C to constant mass.

Water level was measured after gas sampling at DW, SW and SF (when SF had standing water in August and September 2012). At site SF (when there was no standing water in November 2011, May, July and October 2012) and SFC, a 1-m PVC tube was inserted vertically into the soil under the chamber after all monthly gas sampling was complete, allowing two hours for the water level to equilibrate before measuring the level. The water table of site NF was calculated according to the elevation measured by a Global Navigation Satellite System receiver (BLH-L90, Daheng International, China).

Soil water content (SWC) was measured every month after all gas sampling with a Soil Water Sensor (UNI1000, Shunlong, China). Soil/sediment samples (0–30 cm) at site DW, SW, SF and NF were collected at each replicate location in November 2011, except site SFC in October 2012. Fresh soil/sediment samples were used for NH_4^+ and NO_3^- analysis using a discrete analyser (Smartchem 300, AMS, Italy). After air-drying and grinding (passing through a 100 mesh sieve), pH of 1:5 soil-water extractions were measured using a pH meter (IQ160, Hach, USA) while soil total carbon (TC) and nitrogen (TN) was analyzed using an elemental analyzer (vario MACRO cube, Elementar, Germany). Soil bulk density was measured following Chinese national standards NY/T 1121.4-2006 (MAPRC, 2007).

2.4 Statistical analysis

Flux differences were tested using a three-way ANOVA, and then using LSD for multiple comparisons (Table 2 and Fig. 4). One-sample T test was used for testing if the negative fluxes were different from zero. A log10 transformation was used to explore the correlation between N_2O flux and environmental variables (air temperature and soil NO_3^-); where

appropriate, a piecewise function (two segment liner) was calculated (SigmaPlot 11.0, SYSTAT, USA). Spearman's Rank Correlation was used to test for correlations between flux and environmental factors. Figure 5, 6, 7 and Table 3 was made using daily average fluxes to eliminate the influence of not independence of fluxes at different times of day. All the analyses above were performed using IBM SPSS Statistics (version 19.0, IBM, USA). Charts were made using SigmaPlot (version 11.0, SYSTAT, USA).

3 Results

3.1 Environmental characteristics

Precipitation occurred from March to November. The highest rainfall was in July which accounted for one fourth of the total (Fig. 2(a)). Water levels rose rapidly after the summer monsoon rainfall, and then declined after August (Fig. 2(d)). Temperature peaked at summertime (Fig. 2(c)). Diurnal range in temperature was about 10 °C. The non-flooded site was very dry before the rains began (Fig. 2(e)), increasing from a dry condition (only 10% water content) to a moist condition after rain (but never exceeding 35%).

3.2 N_2O fluxes

The mean flux from the littoral zone of the Miyun reservoir was 6.8 μg m^{-2} h^{-1} (0.15 μmol m^{-2} h^{-1}), ranging from –136.6 μg m^{-2} h^{-1} to 381.8 μg m^{-2} h^{-1}. Negative flux was observed at all sampling plots in about one-third of the cases ($n=739$, $p<0.001$). In ANOVA (Table 2), both time of year and position on the transect were statistically significant (both $p<0.001$), but time of day was not significant ($p=0.97$). N_2O emission from the non-flooded area (NF) was 17.0±2.3 μg m^{-2} h^{-1}, which was significantly higher ($p<0.001$) than the other 4 areas. There was no statistical difference ($p=0.91$) between emissions from the seasonal flooded area (SF) and its control site (SFC): fluxes were 4.4±0.7 μg m^{-2} h^{-1} and 4.2±0.7 μg m^{-2} h^{-1} respectively. For SW, SF, SFC and NF, the average emission of non-farmland plots was 2.6 μg m^{-2} h^{-1} but the land cropping maize the sampling summer or the last summer reached 24.0 and 8.4 μg m^{-2} h^{-1} respectively (Fig. 4). Especially high emissions (43.7 μg m^{-2} h^{-1}) were observed on farmland of NF (Fig. 4). Besides SF, where the highest emission occurred in late autumn, other high emissions were observed in the warm season, July and August in particular (Fig. 5).

3.3 Relationships between flux and environmental parameters

Rank correlation analysis was carried out between N_2O flux and environmental parameters, but the coefficients were no higher than 0.38 (Table 3). For more information, correlation analysis was also done separately at each water level. The correlations were different among water levels and higher coefficients were shown between flux and air temperature in several cases (Table 1R). Linear correlations can hide important non-linear features and so scatterplots are also shown, where log10 flux was plotted against air temperature and soil NO_3^- (Fig. 6). As fluxes were often negative (and significantly less than zero, implying a sink for N_2O), we carried out a separate analysis of negative fluxes. Piecewise correlations were found between log10 flux and air temperature (Fig. 6). For positive fluxes, there was a negative correlation ($p=0.03$, $n=65$) when the air temperature was from 5.2 °C to 18.7 °C but a positive correlation ($p<0.01$, $n=175$) when air temperature was from 18.7 °C to 31.1 °C. For negative fluxes, there was a positive correlation ($p<0.01$, $n=43$) when the air temperature was from 5.2 °C to 17.6 °C and a insignificant negative correlation ($p=0.12$, $n=41$) when air temperature was from 17.6 °C to 31.1 °C

We present the relationship between nitrate and N_2O emission. For positive flux, the soil NO_3^- seemed to accelerate N_2O emission when its concentration was higher than 7.1 mg kg^{-1} ($p<0.01$, $n=122$), but it did not influence emission rate when lower than this 'knot point' ($p=0.30$, $n=118$). Piecewise analysis was not done between negative flux and nitrate considering the narrow nitrate concentration (almost no data when soil NO_3^- higher than 10 mg kg^{-1}).

4 Discussion

4.1 N_2O flux

Variations of N_2O fluxes were compared at different spatial and temporal scales (Fig. 4 and Table 2). Significant differences were observed among water levels and sampling months, but not among times of day. Diurnal variation in N_2O flux over lakes and reservoirs has seldom been discussed. However diurnal variation in other aquatic system also seems to be insignificant (Xia et al., 2013). Further research is required on the infrequently-studied diurnal variation in N_2O flux. We may expect soil microbes to respond to temperature, and given a diurnal range in temperature of about 10 ℃ we would have expected a diurnal pattern in the

N_2O flux. We assume that the reason for a lack of response is that the microbial population is mostly deep in the soil/sediment/water system, where temperature variations are much smaller.

The mean flux from the littoral zone of the Miyun reservoir was 6.8 μg m^{-2} h^{-1}, from –136.6 μg m^{-2} h^{-1} to 381.8 μg m^{-2} h^{-1}. Negative fluxes were observed in about one-third of the cases, demonstrating a process of N_2O consumption to be occurring. It is generally acknowledged that under certain conditions the capacity of soil to be a sink for N_2O can, through denitrification, exceed its capacity to emit N_2O (Baggs and Pilippot, 2010). .

How do these fluxes compare to those reported from elsewhere? Our fluxes are comparable to those from the littoral zone of temperate-zone lakes, for example, a shallow lake in Eastern Austria (Soja et al., 2014). However, in most of the cases, our fluxes were lower, as shown by the following comparisons. One similar-latitude lake, Lake Baiyangdian, had nearly 10 times higher N_2O emissions, averaging 58 μg m^{-2} h^{-1} (Yang et al., 2012). Higher emission also been reported in the littoral zone of lower-latitude sites, for example the Three Gorges Reservoir (Table 4). The seriously eutrophic Taihu Lake (latitude: 30°N) had a broader extent ranging from –278 to 2101 μg m^{-2} h^{-1} in the littoral zone (Wang et al., 2007). Greenhouse gas emissions from low latitude ecosystems are found to be higher than the corresponding ecosystems at high latitude because of the temperature effects (Zhu et al., 2013). The average N_2O emission found in the present research was lower than that reported for boreal and Antarctic lakes (Huttunen et al., 2003; Y. Liu et al., 2011). The low N_2O emission of Miyun Reservoir might be the consequence of relatively good water quality or high soil pH (Van den Heuvel et al., 2011).

As for the case of CH_4, N_2O emissions from the littoral zone has been reported to be greater than for the pelagic zone (e.g. Huttunen et al., 2003 and see Table 4).We did not examine N_2O fluxes from the pelagic zone in this research, but we can compare our fluxes with pelagic data from elsewhere, as follows. The N_2O emission in this study is slightly higher than those from five perialpine and alpine reservoirs (1.56 μg m^{-2} h^{-1}) in Switzerland (Diem et al., 2012), while it is much lower than a same-latitude fluvial reservoir (84 μg m^{-2} h^{-1}) located in an agricultural landscape near Indianapolis, USA (Jacinthe et al., 2012b). It should be noted that the comparison between littoral zone and pelagic zone of different reservoirs includes uncertainties, for example differences of elevation, nutrients input and influence of topography on microclimate.

4.2 Relative greenhouse gas effect: comparison with CH_4

Elsewhere, we presented data on methane emissions from this reservoir (M. Yang et al., 2014). The Global Warming Potential (GWP) of N_2O over a 100-year time-span is 298 while CH_4 is 34 (Stocker et al., 2013). We can use the GWPs to calculate the emissions as CO_2-equivalent emissions, and thus compare the warming effect of the two gases. The mean N_2O emission in this study was 2.0 mg CO_2-equivalent m^{-2} h^{-1}. The CH_4 emission was 44.2 mg CO_2-equivalent m^{-2} h^{-1} (M. Yang et al., 2014), which is 22.1 times that of N_2O. This contrasts with our previous findings, where the warming ratio of CH_4:N_2O was 1.5 (Li et al., 2014). But in our earlier report, N_2O variation was investigated with a water recession process. Significant increases (nearly up to 1000 times) were observed after sediment exposure of 5 months. The high emissions may be the result of soil water content declining to 60–90% (Ciarlo et al., 2007). In this research, the soil water content was not in this range at all, and that may have biased the comparison. In general, the flux ratio of CH_4 to N_2O in aquatic environments varies considerably. For example, the CH_4:N_2O ratio of permanent flooded areas at Poyang Lake was 1.1 (Liu et al., 2013) while the ratio was 0.6 for the pelagic zone of a fluvial reservoir in central Indiana (Jacinthe et al., 2012b). In a study which monitored the flux of both littoral and pelagic zone of a temperate lake, the average CH_4:N_2O ratio is 7.2 (Soja et al., 2014). For a freshwater marsh at northeast of China, it was found to be as high as 66.5 (Yang et al., 2013). Although the ratio varies greatly, there is nevertheless a considerable contribution of N_2O emission from aquatic ecosystems to global warming, whose importance may have been somewhat understated in relation to the large CH_4 emission.

4.3 Environmental controls

4.3.1 Flooding

Unlike the specific influence of flooding on CH_4 emission (M. Yang et al., 2014), flooding effects on N_2O emission was not very clear in this study. The N_2O flux of seasonal flooded area SF was as high as its control area SFC which escaped flooding because of higher elevation (Fig. 4). Inundation nearly always causes a drop of N_2O emissions (Yang et al., 2013). Standing water could inhibit N_2O emission through slowing down the diffusive transportation of gas, causing anoxia, activating a different component of the microbiota, leading to the reduction of N_2O to N_2 (Liengaard et al., 2013; Pilegaard, 2013). Our result did not reject those possibilities when looking into the seasonal variation of N_2O flux of seasonal

flooded sites, they did not completely support that hypothesis either (Fig. 5(c)). After flooding, the fluxes of two sites (SF-A and SF-B) were no higher than before flooding and no higher than their control sites. However, a single extraordinary observation showed the highest emission during flooding (Fig. 5(c), SF-C). A somewhat similar result was also observed at an artificial wetland (Hernandez and Mitsch, 2006). An incubation study showed both increasing N_2O emission and stable emission during flooding at different treatments, i.e. N_2O emission of residue-incorporated soils, increased remarkably from the 6th to 30th days of flooding and decreased to lower level than before flooding afterward. However, the N_2O emission of the soils with residues on the surface was stable before and during flooding (Zschornack et al., 2011). It suggested that other factors would influence N_2O emission responses to flooding. Even thought there are uncertainties about the mechanisms, this study implied that flooding introduces a complex set of processes that influence N_2O flux, when compared to non-flooded areas whose fluxes were all more or less coordinated with temperature variation (Fig. 5(a), (b) and (d)).

Besides, floods may influence N_2O production both in the long-term and short-term (Jacinthe et al., 2012a). Quick response of N_2O flux after flooding was showed at a coastal marsh, i.e. N_2O emission decreased in 2.5–5 hours after flooding but then increased to the original level after flooding for 7.5 hours (Sun et al., 2014). The possibility of emissions occurring in discrete pulses, especially by ebullition, should be kept in mind when interpreting results from flux chambers. It also emphasizes the importance of continuous high frequency monitoring to reveal flooding effects with lower uncertainties.

4.3.2 Other environmental conditions

Positive correlations between N_2O emission and temperature were reported in previous studies (e.g. Wang et al., 2014). But in this study we found both positive and negative fluxes, and decided to fit a piecewise regression to the log-transformation data (Fig. 6). This complex and non-linear picture might explain the low coefficients in the correlation analysis (Table 3).

N_2O production is generally caused by several processes, for example denitrification, nitrification, nitrate ammonification and nitrifier denitrification. N_2O consumption has been much less studied (Baggs and Pilippot, 2010). Some studies have found denitrification to be the main contributor in N_2O emission while some others pointed out that several processes occurred simultaneously with a shifting dominance of processes caused by environmental limitations, for instance soil moisture and O_2 availability (Kool et al., 2011; Zhu et al., 2013).

Controlled studies showed that N_2O production via a single process always changes according to temperature, if not exceeded by biotic tolerance (Sierra, 2002; Veraart et al., 2011). Our complex N_2O response to temperature supported the latter notion, i.e. multi-processes occurring and competing during our sampling campaigns. Furthermore, it demonstrated that the response of N_2O production and consumption to temperature was at different rates (Xie et al., 2003). As some chambers within a treatment showed efflux whilst others showed influx, we may presume that the substrate is patchy, over scales of a few metres, reflecting an underlying heterogeneity possibly raised by decaying vegetation.

Negative relationships between N_2O flux and O_2 are reported in both laboratory experiments and field studies (Rosamond et al., 2012; Rubol et al., 2012; Zhao et al., 2014). This is explained by the fact that denitrification, which is activated in anoxic environments, is likely controlling N_2O emissions (Xia et al., 2013). Our present result contradicted those previous conclusions because a significantly positive correlation was observed between N_2O flux and water DO (Fig. 7). N_2O accumulation in the water column has been shown to depend not only on production rate, but also on the extent of N_2O reduction to N_2 by reductase enzymes (Zhao et al., 2014). An incubation study showed that denitrifying activity decreased along with decline of DO concentration, but the N_2O producing activity increased because of less N_2O reduction to N_2 (Senga et al., 2002). Furthermore, Senga's study also pointed out that N_2O produced by nitrification could also be reduced to N_2 via denitrification. That might have happened in our sampling field, i.e. along with increasing of water DO, decreasing of N_2O reduction to N_2 allowing more N_2O to be released at water-air interface, no matter which processes the N_2O was produced. Further study should focus on responses of both N_2O production and reduction to water DO and factors determining which process is the dominance.

Soil NO_3^- is an important substance in N cycle (Butterbach-Bahl et al., 2013). Positive correlations between N_2O flux and nitrate are reported broadly (Soja et al., 2014; Y. Liu et al., 2011; X. L. Liu et al., 2011). It is therefore not surprising to find the highest emission where highest soil NO_3^- occurred. However, in this research when soil NO_3^- was less than the threshold value of 7.1 mg kg^{-1} there was no relationship with NO_3^-. In agricultural studies the NO_3^- concentration are generally much higher, but even then a threshold phenomenon has been reported (Bao et al., 2012). It implied that substrate constrain might be a reason for the weak correlations between N_2O flux and other environmental factors. In the present study, no

significant correlation was showed between N_2O flux and NH_4^+, although NH_4^+ is also important in the N cycle. An N fertilizer experiment in a temperate forest found that the N_2O emissions were only significantly correlated with soil NO_3^- and temperature, but not soil NH_4^+ (Bai et al., 2014). An global review study found that among the five chemical forms of N fertilizer assessed (including NH_4^+), NO_3^- showed the strongest stimulation of N_2O emission, approximately 2 to 3 times higher than the others (Liu and Greaver, 2009).

Based on the above discussion and discussion in a previous paper (M. Yang et al., 2014), the influence of environmental factors on N_2O and CH_4 emission was summarized as follow. The emissions of these two gases are influenced by different factors and in different ways (Table 3), depending on soil conditions, meteorology and vegetation. Methane shows relatively strong correlation with environmental variables while the correlations are always rather weak in N_2O, reflecting the number and complexity of the microbial processes governing the flux of N_2O. The variables likely to be associated with anoxia (soil water depth, soil water content, water DO) were important for both N_2O (see above discussion) and CH_4 (Serrano-Silva et al., 2014) but acted in converse ways. Soil nutrients also influence both of the two gases, but, it seems, through different parameters (Table 3). Different forms of C or N tend to be consistency in soil/sediment, so consistency emission of N_2O and CH_4 along nutrients is expect, but sometimes could be covered by effects of soil water content. Soil water condition in natural environment controls anoxia and influence soil temperature and soil nutrients, implies the fundamental role of soil water level playing in N_2O and CH_4 emission. Therefore, we conclude that water level is the most important factor determining N_2O and CH_4 emission in littoral zone.

4.4 Comparison with farmland

Reclamation of the shore by local farmers, to supplement their income, is not rare. In this research we compared the N_2O emission of natural and farm-related area in the littoral zone. Significant higher emissions were observed at sites cropped with maize in the sampling season or the last growing season. The emission was 24.0 $\mu g\ m^{-2}\ h^{-1}$ and 8.4 $\mu g\ m^{-2}\ h^{-1}$ respectively, while the emission of natural sites was 2.6 $\mu g\ m^{-2}\ h^{-1}$. As discussed in the above section, soil NO_3^- might partly explain the flux difference between farm related land and natural land. Besides, tillage might also influencing N_2O emission through soil aeration (Buchkina et al., 2013).

Reservoirs are being developed, in part, for 'clean energy', and reports of high greenhouse gas emissions from reservoirs have already led some authors to question the 'clean' concept, especially in relation to the mitigation of climate change (Gunkel, 2009). To evaluate the role that reservoirs play in climate change, their greenhouse gas emissions ought to be compared with those of the prior ecosystem (Tremblay et al., 2005). Farmland is one of the several ecosystems which are lost by flooding during reservoir construction in China. In this two compare cases (Table 4), total emission of N_2O and CH_4 in littoral zone was higher than farmland, respectively. The range of soil water content of most farmland soils is relatively narrow and even. Besides rice growing, crops do not tolerate flooding or drought. But soil moisture of the littoral zone is patchy and ranges from flooded to seasonally dry. The littoral zone is therefore precarious in terms of N_2O or CH_4 emissions than farming (Groffman et al., 2009). Even thought the emission of littoral zone was higher, considering its small area and the low emission of pelagic zone, N_2O and CH_4 emission of reservoir is high likely lower than farmland. It's worth noting that N_2O and CH_4 emission of different types of crop might vary the comparison, especially when refers to rice paddy whose CH_4 emission might high enough to result in opposite conclusion. Besides, N_2O emission of farmland was higher than both of littoral zone and pelagic zone, perhaps because of fertilizer application.

5 Conclusions

Finally, we return to our original hypothesis, which was: the littoral zone is a hot-spot of N_2O emissions that is influenced by seasonal changes in the water level. We find that the littoral zone is indeed a hot-spot for N_2O in the summer, especially when the shores of the lake are used for opportunistic farming of maize. But in terms of the overall greenhouse gas budget, the fluxes of N_2O from the littoral zone are not as important as those of CH_4.

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Table 1. Dominant plant species at each plot in different months. DW: deep water site, SW: shallow water site, SF: seasonally flooded site, SFC: 'control site' for seasonally flooded site, NF: non-flooded site. A, B, C indicates sample plot with different vegetation. Species with aerenchyma are denoted [A], species that are emergent are denoted [E].

Site		Nov 2011	May 2012	Jul 2012	Aug 2012	Sep 2012	Oct 2012
DW	A	*Echinochloa olonum*[AE]	*Myriophyllum* sp.		*Trapa*[AE] sp.		
	B	no vegetation					
	C	*Typha angustifolia*[AE]					
SW	A	*Xanthium sibiricum*[E]	*Scirpus planiculmis*[AE]		*Echinochloa colonum*[AE]		
	B	*Setaria viridis*[E]	*Bidens pilosa*[E]		*Echinochloa colonum*[AE]		
	C	*Zea mays*[E]	*Polygonum lapathifolium*[E]				*Typha angustifolia*[AE]
SF	A	*Xanthium sibiricum*	*Cirsium setosum*		*Cirsium setosum*[E]		*Cirsium setosum*
	B	*Setaria viridis*	*Hemarthria altissima*		*Hemarthria altissima*[E]		*Hemarthria altissima*
	C	*Zea mays*	*Polygonum lapathifolium*		*Polygonum lapathifolium*[E]		*Polygonum lapathifolium*
SFC	A	no data			*Cirsium setosum*		
	B	no data			*Hemarthria altissima*		
	C	no data			*Zea mays*		
NF	A	*Xanthium sibiricum*					
	B	*Setaria viridis*	*Artemisia argyi*				
	C	*Zea mays*					

Table 2. ANOVA table to test the effects of water level, sampling month and time of day on N_2O flux. The category of farmland included 4 plots, i.e. SW-C, SF-C, SFC-C and NF-C, which grown maize in the sampling growing season or the last growing season. The category of non-farmland included other 11 spots (see Table 1 for details of vegetation).

	Effect	Type III SS	df	MS	F	p
All	Water level	65,808	4	16,452	25.3	<0.001
	Month	65,546	5	13,109	20.2	<0.001
	Time	918	6	153	0.2	0.965
	Water level * Month	176,351	17	10,374	16.0	<0.001
	Water level * Time	4,901	24	204	0.3	0.999
	Month * Time	7,277	30	243	0.4	0.999
	Waterlevel * Month * Time	31,728	102	311	0.5	1.000
	Error	1,347,885	2073	650		
Non-farmland	Water level	2,982	4	745	5.9	<0.001
	Month	3,525	5	705	5.6	<0.001
	Time	668	6	111	0.9	0.505
	Water level * Month	11,830	17	696	5.5	<0.001
	Water level * Time	3,087	24	129	1.0	0.431
	Month * Time	4,657	30	155	1.2	0.179
	Waterlevel * Month * Time	14,385	102	141	1.1	0.198
	Error	186,701	1485	126		
Farmland (or use to be)	Water level	145,935	3	48,645	48.8	<0.001
	Month	214,645	5	42,929	43.1	<0.001
	Time	1,286	6	214	0.2	0.972
	Water level * Month	490,401	12	40,867	41.0	<0.001
	Water level * Time	6,406	18	356	0.4	0.994
	Month * Time	16,766	30	559	0.6	0.972
	Waterlevel * Month * Time	46,388	72	644	0.6	0.988
	Error	439,735	441	997		

Table 3. Spearman's Rank Correlation (r) between flux and environmental variables, included in the table are data from M. Yang et al. (2014) on the flux of CH_4, collected at the same time as the N_2O. ** indicates significant correlation ($p < 0.01$), * indicates significant correlation ($p < 0.05$). SWC: soil water content, DO: dissolved oxygen, TC: total carbon, TN: total nitrogen. Daily average fluxes were used in the correlation analysis, n is from 84 to 324. #: Data of DW was not included in the analysis since there was no contract sampling of farmland and non-farmland.

	N_2O flux	N_2O flux non-farmland#	N_2O flux farmland#	CH_4 flux	Wind speed	Air temp	Water depth	SWC	Water DO	Biomass	Bulk density	Soil pH	Soil TC	Soil TN	Soil NH_4^+	Soil NO_3^-
N_2O flux	1															
CH_4 flux	-0.10			1												
Wind speed	0.14*	0.06	-0.01	0.03	1											
Air temp	0.19**	0.05	0.38**	0.25**	0.30**	1										
Water depth	-0.02	-0.21**	-0.11	0.75**	0.06	0.16**	1									
SWC	-0.12*	-0.33**	-0.04	0.70**	0.03	0.29**	0.87**	1								
Water DO	0.35**	0.04	0.14	-0.28**	0.43**	-0.15	0.24**	0.00	1							
Biomass	-0.08	0.11	0.11	-0.26**	-0.15**	-0.34**	-0.38**	-0.52**	-0.48**	1						
Bulk density	0.00	0.17*	0.13	-0.53**	-0.01	-0.05	-0.78**	-0.67**	-0.26**	0.35**	1					
Soil pH	0.08	0.21**	0.19	-0.17**	-0.02	-0.03	-0.25**	-0.18**	-0.14	0.06	0.35**	1				
Soil TC	-0.04	-0.06	-0.08	0.62**	0.01	0.05	0.81**	0.74**	0.13	-0.35**	-0.77**	-0.26**	1			
Soil TN	0.03	-0.01	-0.06	0.56**	0.03	0.05	0.76**	0.67**	0.15	-0.33**	-0.73**	-0.21**	0.96**	1		
Soil NH_4^+	0.01	-0.13	0.03	0.18**	-0.14*	0.02	0.06	0.23**	-0.21**	-0.16**	-0.12*	-0.02	0.08	0.06	1	
Soil NO_3^-	0.25**	0.09	0.25*	-0.02	0.04	-0.01	0.09	0.10	0.28**	-0.07	-0.20**	0.27**	0.17**	0.19**	-0.11*	1

Table 4. Comparison of N_2O and CH_4 emission from reservoir and farmland (both expressed as CO_2 equivalent, see text). Flux was transformed into CO_2 equivalent according to the Global Warming Potential (Stocker et al., 2013), i.e. 1 N_2O=298 CO_2, 1 CH_4=34 CO_2. *: The N_2O flux equalled 0.87 mg CO_2 m^{-2} h^{-1} while CH_4 flux equalled 60.2 mg CO_2 m^{-2} h^{-1} when excluded farmlands, i.e. SW-C, SF-C, SFC-C and NF-C, which grown maize in the sampling growing season or the last growing season (flat land along water edge of Miyun reservoir always be used for opportunistic cropping by local farmer, more information see section of study area). #: Just SFC-C and NF-C was used for calculation, where grew maize the whole sampling time. §: Unpublished data. Hubei is the province where part of the Three Gorges Reservoir is situated. Beijing is the city which includes the Miyun Reservoir. Maize, rice and wheat are the first three crops in terms of area in China.

	Study area		N_2O (mg CO_2 m^{-2} h^{-1})	CH_4 (mg CO_2 m^{-2} h^{-1})	Sum (mg CO_2 m^{-2} h^{-1})	Data source
Reservoir	Three Gorges Reservoir	littoral zone	9.2	227.8	237	(Chen et al., 2010; Chen et al., 2009)
		pelagic zone	4.2	8.8	13	(Zhu et al., 2013; Chen et al., 2011a)
	Miyun Reservoir	littoral zone	2.0*	44.2*	46.2	This study; (M. Yang et al., 2014)
		pelagic zone	No data	10.2	10.2	(Yang et al., 2011)
Farmland	China-IPCC		2.5–16.7	ND	2.5–16.7	(Xu et al., 2014; Smith et al., 2002)
	Hubei-DNDC		26.8	85	111.8	(Li et al., 2003)
	Typical farmland near Three Gorges Reservoir-observed	rice	24.1	100.6	124.7	(Zhang et al., 2012)
		rice and rape	33.7	47.6	81.3	(Zhang et al., 2012)
	Beijing-DNDC		17.9	6.8	24.7	(Li et al., 2003)
	Typical farmland near Miyun Reservoir-observed	wheat	4.8	0.4	5.2	(Hu et al., 2013)
		maize	24.1	0.5	24.6	(Hu et al., 2013)
		maize	9.1#	-0.3#§	8.8	This study

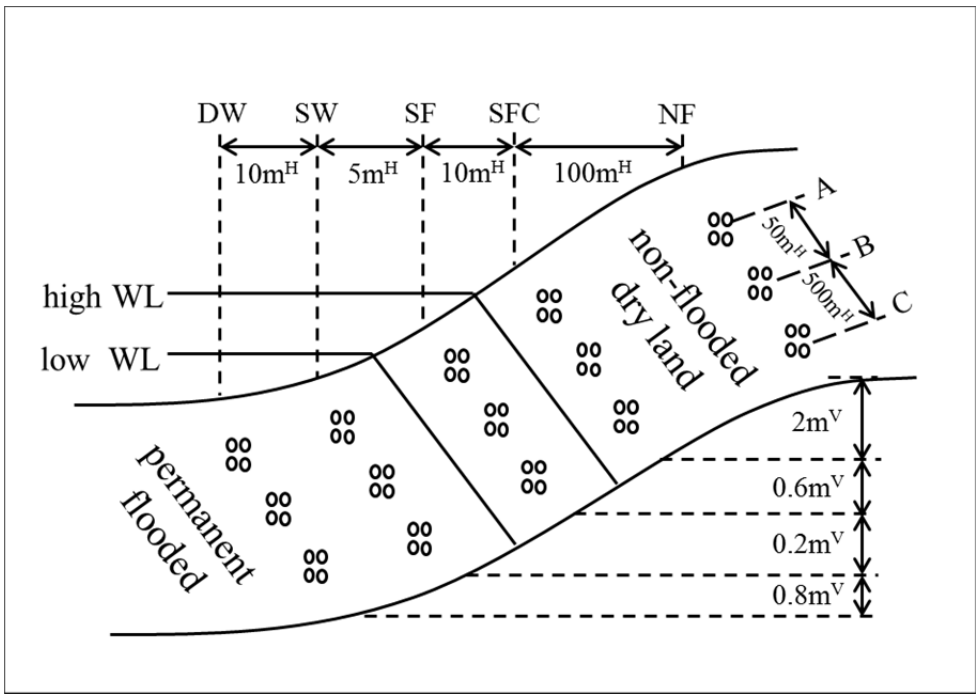

Figure 1. Experimental design. WL: water level. The difference between high WL and low WL was caused by summer flooding. m^H indicates meters in horizontal; m^V indicates meters in vertical. The sites are grouped at different heights. DW: deep water site; SW: shallow water site; SF: seasonally flooded site; SFC: 'control site' for the seasonally flooded site, which had similar vegetation and soil moisture as site SF before it was flooded; NF: non-flooded site, which flooded one time per several years and not flooded in the sampling year. A, B and C denote samples from different vegetation types within each height band, species details see Table 1. There were 15 plots in total, four replicates in each plot, repeatedly sampled six times in the year to cover different seasons and covering the transition in and out of the flooding season. Also to capture diurnal variation, plots were repeatedly sampled seven times per day . For more details on water depth and other environmental parameters, see Fig. 2 and Fig. 3.

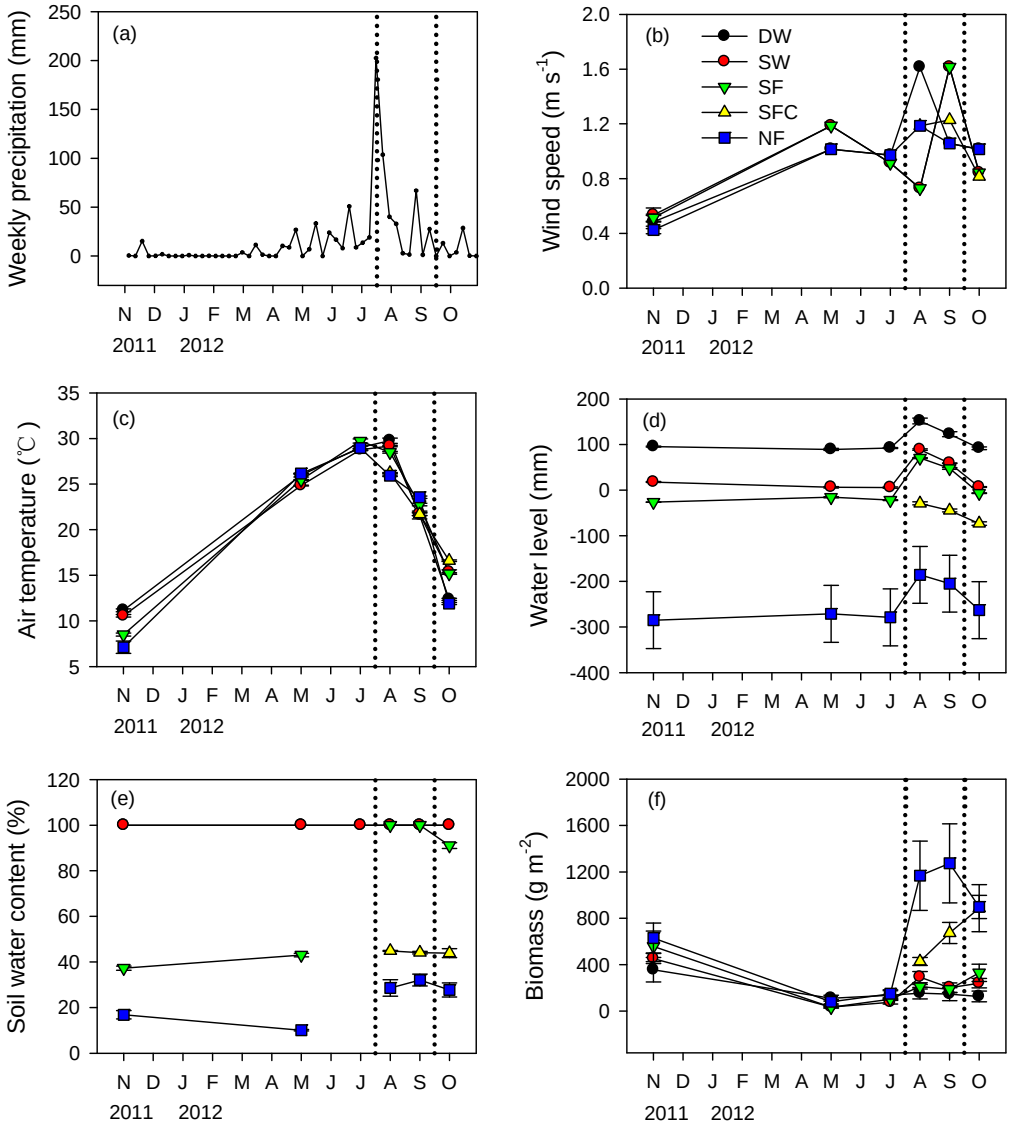

Figure 2. Environmental characters (Mean±SE) of each sampling area. Some SE bars are not visible. Days between dotted lines were the high water level period and thus the seasonal flooded site (SF) was under water. DW: deep water site; SW: shallow water site; SF: seasonally flooded site; SFC: 'control site' for the seasonally flooded site; NF: non-flooded site. There was no soil water content data for July because of instrument malfunction.

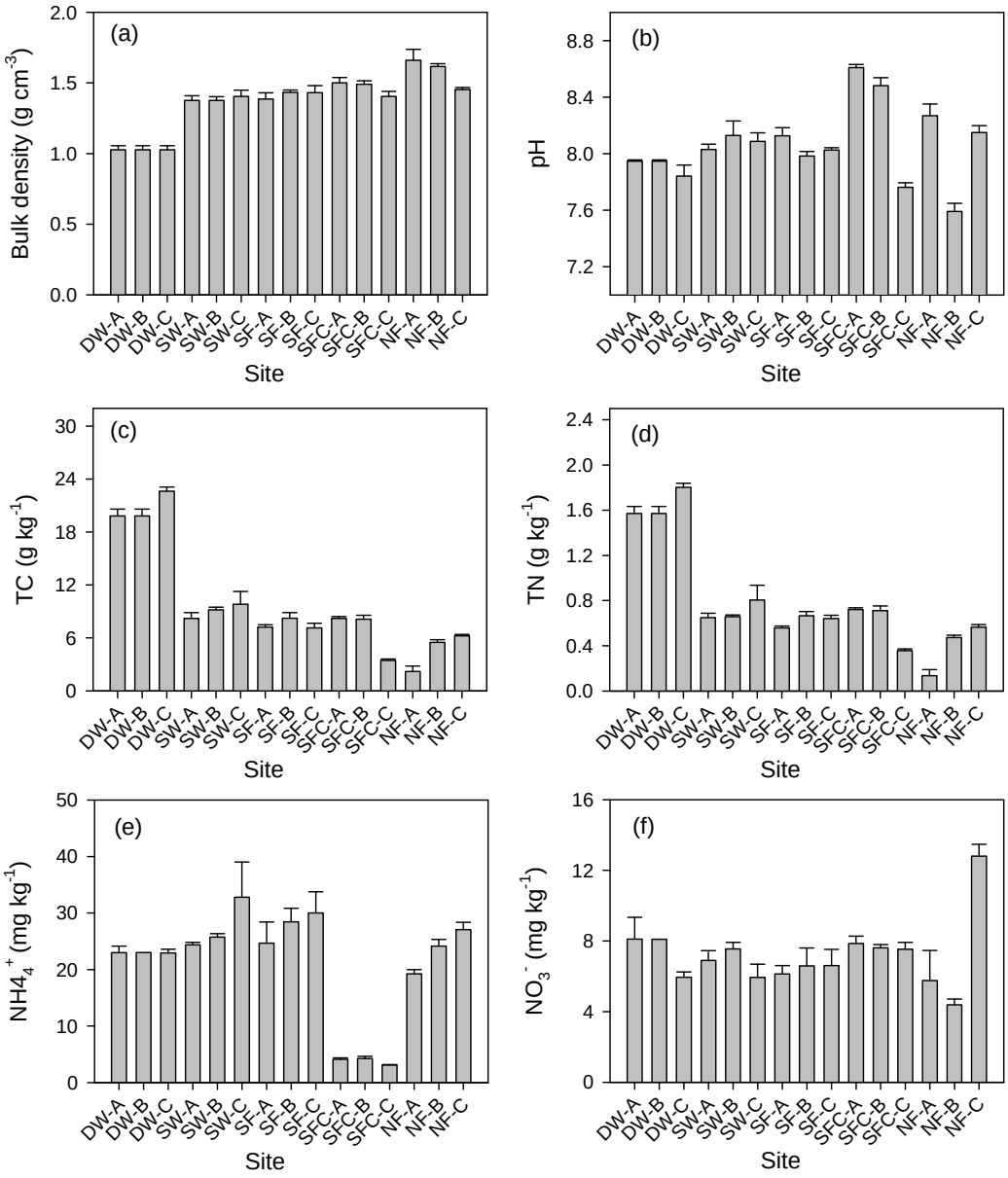

Figure 3. Physicochemical properties (Mean±SE) of soil/sediment of each site. Some SE bars are not visible because they are too small. DW: deep water site; SW: shallow water site; SF: seasonally flooded site; SFC: 'control site' for the seasonally flooded site; NF: non-flooded site. A, B and C denote samples from different vegetation types within each height band.

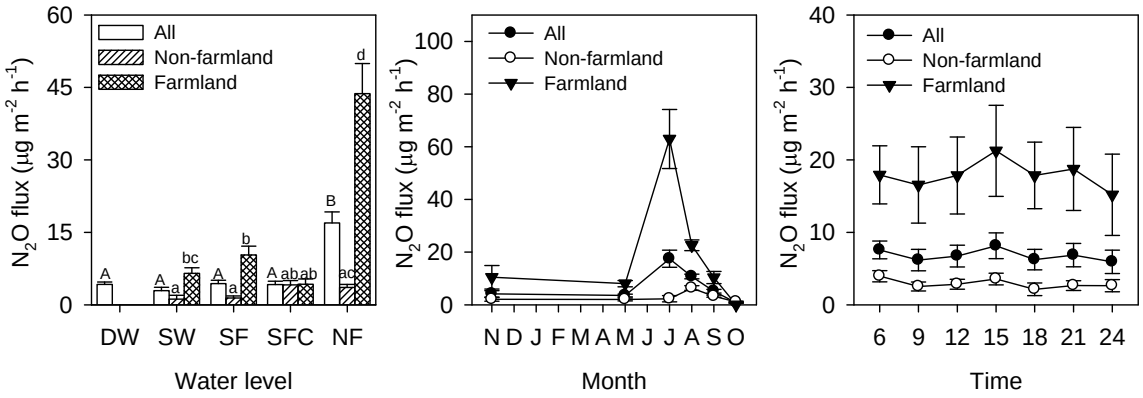

Figure 4. N_2O flux (Mean±SE) at different water levels, months and times of day. Farmland included 4 plots, i.e. SW-C, SF-C, SFC-C and NF-C, which grew maize in the sampling growing season or the last growing season. Non-farmland included other 11 spots (see Table 1 for details of vegetation). DW: deep water site; SW: shallow water site; SF: seasonally flooded site; SFC: 'control site' for the seasonally flooded site; NF: non-flooded site. Bars with different letters indicate a significant difference at $p<0.05$. Difference analysis of bars with capital letters and small letters was done separately.

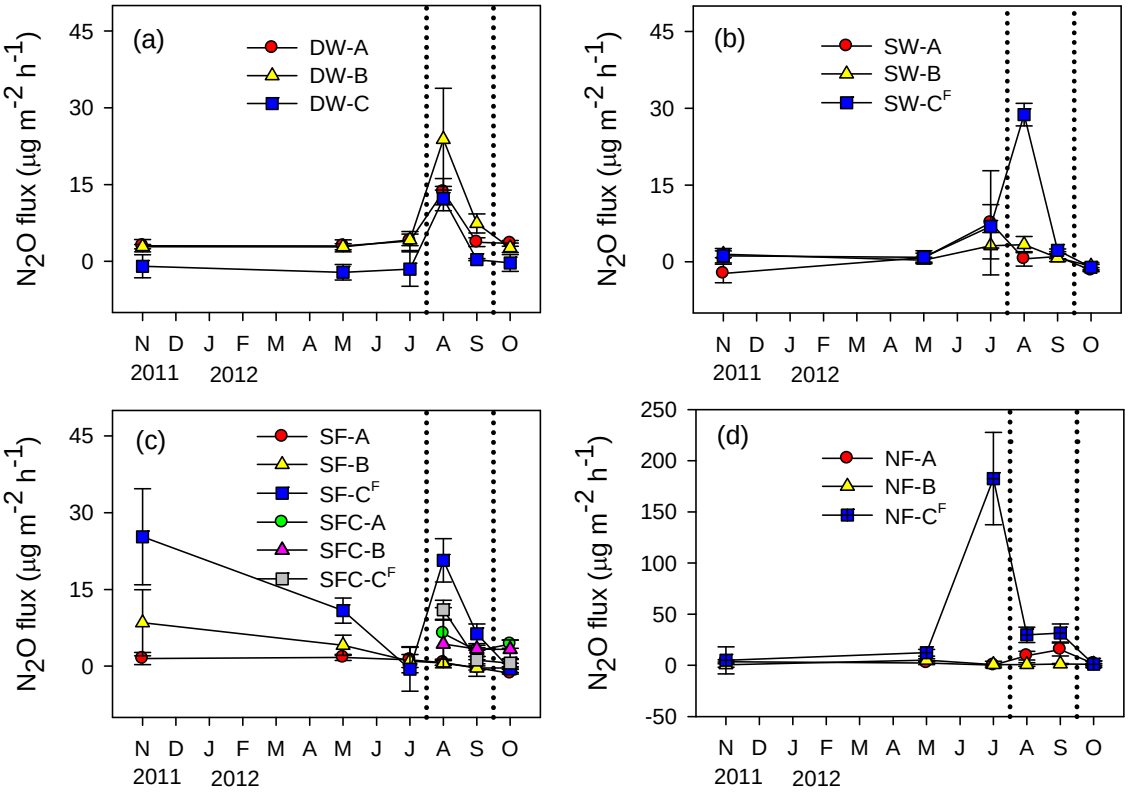

Figure 5. Monthly N_2O flux (Mean±SE) of each site. Days between dotted lines were the high water level period and thus the seasonal flooded site (SF) was under water. DW: deep water site; SW: shallow water site; SF: seasonally flooded site; SFC: 'control site' for the seasonally flooded site; NF: non-flooded site. A, B and C denote samples from different vegetation types within each height band. Superscript F indicates farmland during the whole/part sampling time.

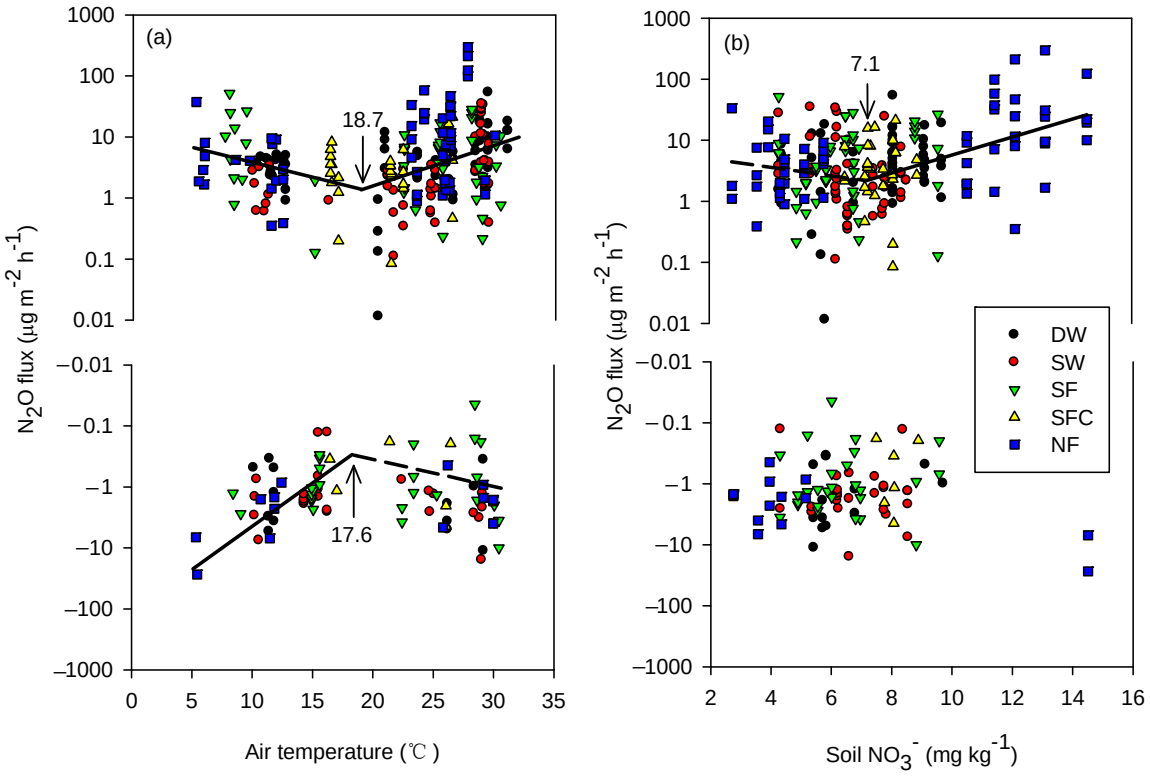

Figure 6. Relationship between flux, air temperature and soil NO_3^-. DW: deep water site; SW: shallow water site; SF: seasonally flooded site; SFC: 'control site' for the seasonally flooded site; NF: non-flooded site. The result of piecewise correlation was plotted using flux data after log10 transformation. Dashed lines indicate insignificant correlations while solid lines indicate significant correlations. See text for details.

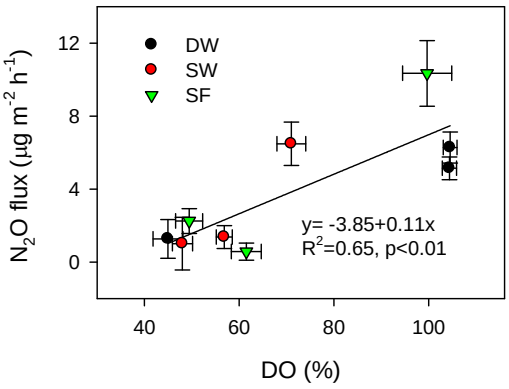

Figure 7. Relationship between flux and water DO (Mean±SE). DW: deep water site; SW: shallow water site; SF: seasonally flooded site.