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# Effect of continued nitrogen enrichment on greenhouse gas emissions from a wetland ecosystem in the Sanjiang Plain, Northeast China: A 5 year nitrogen addition experiment

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[1] Mounting evidence supports that wetland ecosystems, one of the largest carbon pools on the earth, are exposed to ample nitrogen (N) additions due to atmospheric deposition or N loading from upstream agricultural fertilizer application. However, our understanding of how N enrichment affects the fluxes of greenhouse gases (GHGs) in wetlands is weak. A 5 year N addition experiment was conducted to examine the responses of $CH_4$ and $N_2O$ fluxes as well as ecosystem respiration from wetlands in the Sanjiang Plain, Northeast China, through 2005 to 2009. Four levels of N addition (control, 0 kg N $ha^{-1}$ $yr^{-1}$; low-level, 60 kg N $ha^{-1}$ $yr^{-1}$; medium-level, 120 kg N $ha^{-1}$ $yr^{-1}$; high-level, 240 kg N $ha^{-1}$ $yr^{-1}$) were designed in this study. Overall, our results show that medium and high levels of N addition increased ecosystem respiration by 28% and 69% ($P < 0.05$), respectively, while low-level N addition has no effect on ecosystem respiration ($P > 0.05$). High-level N fertilization exerted stronger effects on ecosystem respiration in the initial year than the following years. It indicated that the effects of high-level N fertilization on $CO_2$ might be overestimated by short-term observations. High-level N fertilization increased $N_2O$ emissions by 396% over the 5 years ($P < 0.05$), but the low- and medium-level-N addition did not exert any apparent effect on $N_2O$ emissions ($P > 0.05$). $N_2O$ emission under high-level N addition in the first and fifth years showed stronger pronounced responses to N addition compared with that from the third and fourth years, indicating the importance of long-term field observation. Over the 5 years, however, the low and medium-level N addition showed no effect on $N_2O$ emissions. The four levels of N addition exerted no effect on $CH_4$ emissions ($P > 0.05$). Furthermore, the relationship between GHGs and soil temperature or water table depth varied among different plots and experimental time. Our findings highlighted the importance of gas species, experimental time, and the amount of fertilizer N with regard to the responses of GHG emissions to N fertilization.

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## 1. Introduction

[2] In recent decades, atmospheric nitrogen (N) deposited onto terrestrial ecosystems has increased more than three-fold, primarily due to anthropogenic activities related to fossil fuel combustion and artificial fertilizer production and consumption [*Galloway et al.*, 2004, 2008; *Schlesinger*, 2009; *Pan et al.*, 2012]. N fertilization could stimulate plant growth [*Berendse et al.*, 2001], change species composition [*Xia and Wan*, 2008], and alter soil carbon dynamics [*Burton et al.*, 2004] and the main greenhouse gas (GHG) fluxes including $CO_2$, $CH_4$, and $N_2O$ between ecosystems and the atmosphere [*Pregitzer et al.*, 2008; *Liu and Greaver*, 2009; *de Vries et al.*, 2009; *Lu and Tian*, 2013].

[3] Wetland ecosystem holds higher soil carbon density as compared to other ecosystem types [*Post et al.*, 1982] and plays a critical role in modifying the atmospheric $CO_2$ concentrations [*Treat et al.*, 2007; *Bonneville et al.*, 2008; *Song et al.*, 2011]. More importantly, wetlands are among the primary atmospheric $CH_4$ sources, accounting for 20–39% of the annual global $CH_4$ budget [*IPCC*, 2007]. It is also well reported that wetland ecosystems provide an important natural source of $N_2O$ to the atmosphere [*Hadi et al.*, 2000; *Hernandez and Mitsch*, 2006]. Currently, wetlands, like other

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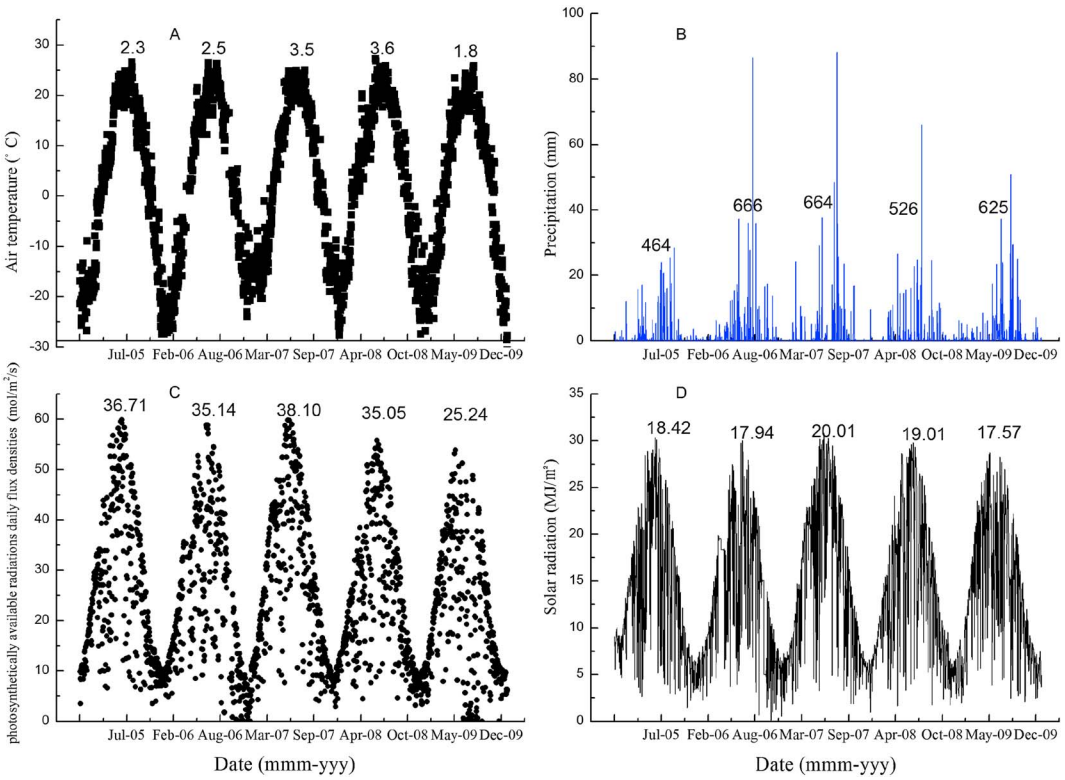


**Figure 1.** Meteorological characteristics during 2005–2009 in the Sanjiang Plain Marshland, Northeastern China.

natural systems, are experiencing mounting N loading due to increased atmospheric deposition, agricultural inputs, fossil fuel combustion, and other anthropogenic activities [*Bridgham et al*., 1995; *Keller et al*., 2004; *Schlesinger*, 2009], and thus the role of N fertilization in controlling GHG emissions to atmosphere from wetland ecosystems is important.

[4] It has been well verified that N addition to wetland ecosystems alters soil physical properties [*Aerts and de Caluwe*, 1999; *Keller et al*., 2005], microbial communities [*Keller et al*., 2005; *Banger et al*., 2012], and vegetation communities [*Chapin et al*., 2004; *Keller et al*., 2005], and thus resulted in altered biogenic flux of the three main GHGs. *Aerts and de Caluwe* [1999] reported that N supply led to pH reduction and to lower $CO_2$ emission, especially in the low N peat soil. Previous studies reporting the effects of N additions on GHG balance, however, have been mostly conducted over relatively short periods, often within the first 1 or 2 years after N was added [*Ding et al*., 2004; *Zhang et al*., 2007a, 2007b, 2007c], which might bias the estimated ecosystem responses in terms of carbon assimilation, respiration, $CH_4$ exchange, and $N_2O$ release.

[5] Reductions in the effects of long-term N fertilization on soil carbon releases were observed in forests, as compared to short-term N fertilization [*Bowden et al*., 2004; *Burton et al*., 2004]. In a meta-analysis, *Liu and Greaver* [2009] also emphasized the necessity in long-term effect of multiple levels of N enrichment on GHG emissions. What about the differences in the effects of long-term N fertilization on GHG fluxes from wetland ecosystems compared to short-term N fertilization? *Keller et al*. [2005] reported that six years of N fertilization had no effect on rates of $CH_4$ production or $CH_4$ consumption in an intermediate fen in northern Minnesota, due to that the microbial communities responsible for soil carbon cycling were tolerant to wide ranges of nutrient concentrations and the resulting pH levels. However, the results from *Keller et al*. [2005] were based on intact peat cores with short-term laboratory incubations rather than long-term continuous field observation. Moreover, a number of experiments only examine the effect of N enrichment on individual flux of $CO_2$, $CH_4$, and $N_2O$, instead of that on the concurrent balance of these three GHGs [*Ding et al*., 2004; *Zhang et al*., 2007a, 2007b, 2007c; *Bodelier*, 2011; *Banger et al*., 2012]. *Ellen et al*. [2012] reported that N fertilization does increase the production of $CH_4$, though there was no effect on $CO_2$ and $N_2O$ fluxes of soil from plots in a tidal freshwater wetland, but the experiment was conducted by anaerobic lab incubations.

[6] In China, current N deposition rates are close to or even exceed those in Europe and the United States [*Lu and Tian*, 2007, *Lu et al*. 2012; *Pan et al*., 2012], and it ranked as the top consumer of N fertilizer during 2007–2008, accounting for 32% of global N consumption [*Heffer*, 2009]. Long-term experiments with multiple levels of N fertilization on the concurrent three GHG balance in China's wetland ecosystems are highly essential for better understanding and predicting the impacts of atmospheric N enrichment on $CO_2$, $CH_4$, and $N_2O$ fluxes from natural wetland ecosystems. The Sanjiang Plain, Northeast China, encompassing the largest freshwater wetland in China, has experienced intensive cultivation over the past 50 years [*Zhao*, 1999]. More and more marshes are being drained for conversion to agricultural production, while the remaining marshes often receive leached N through cultivation activities and atmospheric deposition [*Zhang et al*., 2005].

**Table 1.** Soil Properties of the Study Site in the Sanjiang Plain, Northeast China

| Wetland Type | Depth (cm) | SOC (g/kg) | Total Nitrogen (g/kg) | Total Phosphorus (g/kg) |
|---|---|---|---|---|
| *Calamagrostis angustifolia* | 0–10 | 163.58 ± 7.86 | 7.00 ± 0.32 | 1.11 ± 0.06 |
| | 10–20 | 94.72 ± 5.76 | 4.40 ± 0.29 | 0.74 ± 0.03 |

[7] In the present study, we conducted a 5 year N fertilization experiment, which aims to (1) determine the effects of 5 year experimental N additions on the exchange of $CH_4$ and $N_2O$ and ecosystem respiration in the natural freshwater wetland in the Sanjiang Plain, (2) to evaluate the interannual variations of gas emissions caused by different N input levels, and (3) to examine the relationship between GHG fluxes and related environmental factors, such as soil temperatures and water table depths. We hypothesized that stimulation or inhibition in GHG emissions under N fertilization would be observed based on the 5 year observation; however, the effects of N fertilization would be temporally variable and would not be consistent across gas species and plots with different N input levels.

## 2. Material and Methods

### 2.1. Site Description

[8] The study site is located at the Sanjiang Experimental Station of Wetland Ecology Chinese Academy of Sciences (47°35′N, 133°31′E, 56 m above sea level), in the Sanjiang Plain, Northeast China. The Sanjiang Plain holds freshwater wetland with area of approximately 10,400 $km^2$ [*Zhao*, 1999]. The site-specific weather observations showed that the mean annual temperature is 1.9°C, and mean annual precipitation is around 600 mm during 1981–2004 [*Guo et al*., 2010]. As shown in Figure 1, the averaged annual temperatures were 2.3, 2.5, 3.5, 3.6, and 1.8°C from 2005 to 2009, respectively. It indicated that except the year of 2009, the other 4 years showed much warmer than long-term mean temperature (1981–2004). The annual precipitations for the period of 2005–2009 were 464, 666, 664, 526, and 625 mm, indicating that both 2005 and 2008 were dry years and other 3 years were wet years. The annual mean photosynthetically available radiations (PAR) daily flux densities were 36.71, 35.14, 38.10, 35.05, and 25.24 mol $m^{-2}$ $s^{-1}$, and the annual average solar radiations were 18.42, 17.94, 20.01, 19.01 and 17.57 MJ/$m^2$ from 2005 to 2009. PAR daily flux densities and solar radiations changed very little year by year during 2005 and 2008, but showed the lowest values in 2009. More detailed information about air temperature, rainfall, PAR daily flux densities, and solar radiation are shown in Figure 1. There are two dominant species in the Sanjiang wetland, *Calamagrostis angustifolia* and *Carex lasiocarpa*, and the latter will be more abundant as the standing water depth increases. The site was selected to represent the typical, seasonal waterlogged marsh plant communities, *Calamagrostis angustifolia*. Before the start of the experiment, three replicates of soil from 0 to 10 cm depth and 10 to 20 cm depth were investigated in the study region at the beginning of growing season in 2004. More detailed information on soil properties in the study site is shown in Table 1.

### 2.2. Field Experiment

[9] Twelve plots of 1 m × 1 m were selected on a very flat ground with a *Calamagrostis angustifolia* dominated community. Each sampling plot was isolated by a 1 m buffer zone and an enclosed PVC board, which prevented lateral movement of the added N and its influence to the surrounding area. Inside the PVC frame, a stainless steel base (50 cm × 50 cm) with a water groove was installed into the soil to ensure an airtight connection with the chamber. We built boardwalks around the chamber to avoid human disturbance to the sampling.

[10] Since 2005, N was added as ammonium nitrate ($NH_4NO_3$) during the growing season, as $NH_4NO_3$ has been widely used as N fertilizer in this region after land-use change. Twelve plots (1 m × 1 m) were randomly split into four treatment groups, where the annual N additions total to 0 kg N $ha^{-1}$ $yr^{-1}$, 60 kg N $ha^{-1}$ $yr^{-1}$, 120 kg N $ha^{-1}$ $yr^{-1}$, and 240 kg N $ha^{-1}$ $yr^{-1}$ for control (N0), low-level (N1), medium-level (N2), and high-level N fertilization (N3), respectively. N1 (60 kg N $ha^{-1}$ $yr^{-1}$) was comparable to the sum of agricultural inputs (58 kg N $ha^{-1}$ $yr^{-1}$) [*Zhang et al*., 2007a] and atmospheric wet N deposition (7.6 kg N $ha^{-1}$ $yr^{-1}$) around this region [*Sun*, 2007], while the medium- and high-level N addition were used to study the response of GHGs to N fertilization that may occur in this region in the future [*Zhang et al*., 2007a]. In each year, N fertilizer was divided into nine equal doses, mixed with 1 L surface marsh water and applied biweekly from May to September. At the same time, the control plots received 1 L surface marsh water without N fertilizer. The solution was sprayed over the plants, and there was a deionized water rinse afterward. There was one chamber per plot, i.e., three plots with three chambers for one treatment, and the mean value was analyzed. Observation was conducted once a week from early May to late September in 2005, 2007, 2008, and 2009. Sometimes, observations

**Table 2.** Average Values of Soil Temperature at 5 cm Depth, Water Table Depth, and Aboveground Biomass Under Different N Addition Levels[a]

| | Soil Temperature at 5 cm Depth | Water Table Depth | Aboveground Biomass |
|---|---|---|---|
| N1 | 14.53 ± 0.67a | 2.61 ± 0.64a | 250.23 ± 16.74a |
| N2 | 13.18 ± 0.54a | 3.30 ± 0.73a | 322.92 ± 31.47a |
| N3 | 13.19 ± 0.57a | 3.51 ± 0.73a | 365.81 ± 32.16a |
| N4 | 13.48 ± 0.57a | 2.72 ± 0.65a | 626.87 ± 86.18b |

[a]Average is (mean ± SE); the unit of soil temperature is °C; the unit of water table depth is centimeter; the unit of aboveground biomass is g $m^{-2}$. There was significant difference among the averaged values with different lower-alpha after the averaged values, whereas no significant difference was found among the averaged values with the same lower-alpha after the averaged values.

**Table 3.** Results of Two-Way ANOVAs on the Effects of N Addition (N), Years (Y), and Their Interactions on Soil Temperature at 5 cm Depth, Water Table Depth and Aboveground Biomass

| | Soil Temperature at 5 cm Depth | | | Water Table Depth | | | Aboveground Biomass | | |
|---|---|---|---|---|---|---|---|---|---|
| | d.f. | *F* | *P* | d.f. | *F* | *P* | d.f. | *F* | *P* |
| N | 3.00 | 0.00 | 1 | 3.00 | 0.46 | 0.71 | 3.00 | 65.39 | <0.001 |
| Y | 2.00 | 0.72 | 0.49 | 2.00 | 13.89 | <0.001 | 2.00 | 9.67 | <0.001 |
| N × Y | 6.00 | 0.00 | 1 | 6.00 | 0.07 | 1 | 6.00 | 24.31 | <0.001 |

were rescheduled because of unpredictably extreme weathers, such as heavy rainfall or strong wind. In 2006, N addition was applied as the other years, while observations ceased.

### 2.3. Measurement Method

[11] The gas samples were usually taken at 9:00 A.M. at local time using static dark chamber and gas chromatography techniques [*Wang and Wang*, 2003; *Song et al.*, 2009]. The static chamber was made of stainless steel and consisted of two or three parts. Before measurement, one square collar (length × width × height = 0.5 m × 0.5 m × 0.2 m), serving to support the sampling chamber, was inserted directly into the soil with 5 cm exposed above the soil/water surface during the entire experiment. One square box (without bottom, length × width × height = 0.5 m × 0.5 m × 0.5 m) was put above the collar during gas sampling. When the plant height exceeds 50 cm, a removable collar (length width × height = 0.5 m × 0.5 m × 0.2 m) was added between the collar in soil and the cover box to protect the plant. We installed a fan in the sampling chamber to promote air mixing. Gas samplings were lasted half an hour for each time; four gas samples were taken at 10 min intervals. Meanwhile, air temperatures inside and outside the chambers were recorded using a thermometer sensor. The gas samples were stored in plastic syringes with three valves and measured with a modified gas chromatograph (Agilent 4890D, Agilent Co., SantaClara, CA, USA) equipped with a flame ionization detector and an electron capture detector. We analyzed the $CH_4$, $CO_2$, and $N_2O$ simultaneously once after coming back from the field, and the gas samples were stored in syringes less than 12 h before being measured. $N_2$ was used as the carrier gas with a flow rate of 30 mL $min^{-1}$. The $CO_2$, $CH_4$, and $N_2O$ emissions were calculated from the linear changes in chamber gas concentration with an average chamber temperature (air temperature in the chamber). When the $R^2$ of the linear regression of the $CO_2$, $CH_4$, and $N_2O$ concentrations over time was lower than 0.9, sample sets were rejected. The units were expressed as mg C $m^{-2}$ $h^{-1}$ for $CO_2$ and $CH_4$, and mg N $m^{-2}$ $h^{-1}$ for $N_2O$. More detailed information about gas sampling was described by *Song et al.* [2009]. GHG data in 2005 are selectively cited from *Zhang et al.* [2007a, 2007b, 2007c], and we kept the sampling date and total sampling points close to those in other years. The surface water table was measured using a ruler near the chamber in each plot in 2007, 2008, and 2009, and soil temperature at 5 cm near each plot was measured with bent stem thermometers. Aboveground biomass was estimated by clipping living biomass. All living plant tissues were harvested from every 50 cm × 50 cm quadrat in each plot at the end of the growing season in 2007 and 2008, respectively. Living plant aboveground tissues were separated from dead tissues, oven-dried at 70°C for 48 h, and weighed [*Xia et al.*, 2009]. All the harvested biomass was returned to the quadrats with random distribution after the measurement. The aboveground biomass data in 2005 are cited from *Zhang et al.* [2007a, 2007b]. Daily information on air temperatures and rainfall were automatically recorded by a microclimate monitoring system throughout the study period.

### 2.4. Dependence of Ecosystem Respiration on Soil Temperature

[12] Soil temperature and respiration data from 2007 to 2009 (n = 64) were fitted to exponential functions given in equation (1) to describe the dependence of ecosystem respiration on soil temperature [*Maier and Kress*, 2000].

$$\mathrm{ER} = a \times e^{bt} \tag{1}$$

where ER and $t$ are ecosystem respiration and soil temperature, respectively, and $a$ and $b$ are regression coefficients. To accurately quantify the sensitivity of ecosystem respiration to soil temperatures, we used soil temperature coefficient ($Q_{10}$) as the index to show the increase in ecosystem respiration when the soil temperature changed. The $Q_{10}$ is equal to the change of ecosystem respiration for temperature increase of every 10°C. The temperature sensitivity ($Q_{10}$) of ecosystem respiration was calculated as follows [*Maier and Kress*, 2000]:

$$Q_{10} = \exp(b \times 10) \tag{2}$$

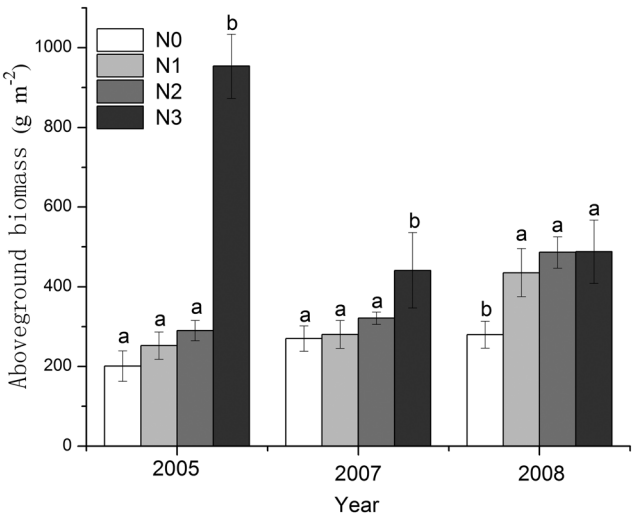


**Figure 2.** The aboveground biomass under different N addition levels from the freshwater wetland in the Sanjiang Plain, Northeast China.

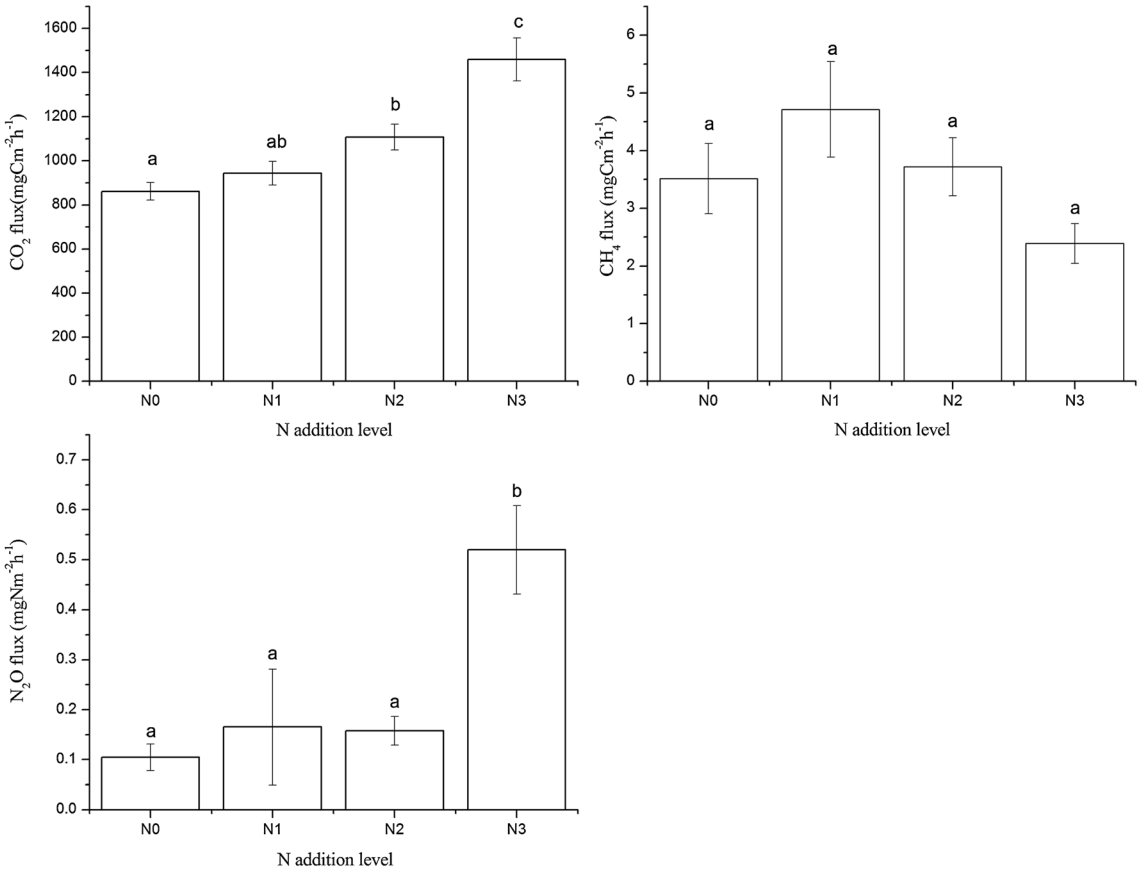


**Figure 3.** Overall estimates of GHGs flux from marshes under different N addition levels for 2005 to 2009. Error bars represent ± SE (n = 3). Means with different lowercase letters in the same column are significantly different at $P < 0.05$. N0, 0 kg N $ha^{-1}$ $yr^{-1}$; N1, 60 kg N $ha^{-1}$ $yr^{-1}$; N2, 120 kg N $ha^{-1}$ $yr^{-1}$; N3, 240 kg N $ha^{-1}$ $yr^{-1}$.

### 2.5. Statistical Analysis

[13] The SPSS 11.5 and origin 8.0 statistical packages were used for statistical analysis. All the data were tested for normality using the Levene's test prior to analysis. Before statistical analysis, all non-normal data were transformed prior to analysis. Two-way analysis of variance (ANOVA) was used to examine the effects of N addition, year, and their possible interactions on soil temperature, water table depth, aboveground biomass, and ecosystem GHG fluxes. If there is significant interannual variability (year effect $P < 0.05$), one-way ANOVA was used to examine N addition effects on soil microclimate, aboveground biomass, and ecosystem GHG fluxes over the growing seasons in 2005, 2007, 2008, and 2009, respectively, using a Fisher's least significant difference test. The Spearman correlation tests were used to analyze the relationship between GHG emissions and water table depth or soil temperature from 2007 to 2009. Linear and exponential analyses were used to examine the relationships between soil temperature and ecosystem respiration from 2007 to 2009. In analyses where $P < 0.05$, the factor relationship tested and the comparisons were considered statistically significant.

## 3. Results

### 3.1. Soil Microclimate and Plant Biomass

[14] As shown in Table 2, the average soil temperatures at 5 cm depth were 14.53 ± 0.67°C, 13.18 ± 0.54°C, 13.19 ± 0.57°C, and 13.48 ± 0.57°C for N0, N1, N2, and N3, respectively, while water table depth were 2.61 ± 0.64 cm,

**Table 4.** Results of Two-Way ANOVAs on the Effects of N Addition (N), Years (Y), and Their Interactions on $CO_2$, $CH_4$, and $N_2O$ Emissions

| | $CO_2$ | | | $CH_4$ | | | $N_2O$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | d.f. | *F* | *P* | d.f. | *F* | *P* | d.f. | *F* | *P* |
| N | 3.00 | 11.13 | <0.001 | 3.00 | 4.86 | <0.001 | 3.00 | 19.64 | <0.001 |
| Y | 3.00 | 9.40 | <0.001 | 3.00 | 26.96 | <0.001 | 3.00 | 11.88 | <0.001 |
| N × Y | 9.00 | 4.14 | <0.001 | 9.00 | 1.39 | 0.19 | 9.00 | 2.25 | 0.02 |

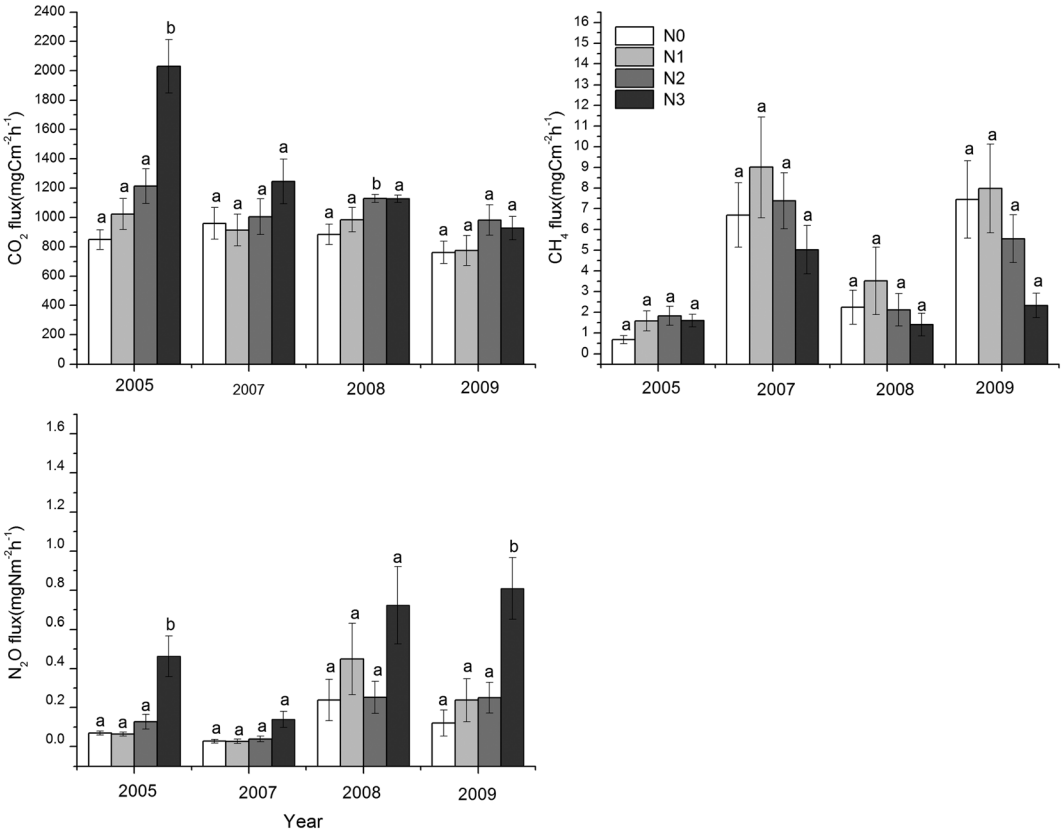


**Figure 4.** GHG emissions during the growing season from 2005 to 2009 in the control and nitrogen addition treatments. Error bars represent ± SE (n = 3). Means with different lowercase letters in the same column are significantly different at $P < 0.05$.N0, 0 kg N $ha^{-1}$ $yr^{-1}$; N1, 60 kg N $ha^{-1}$ $yr^{-1}$; N2, 120 kg N $ha^{-1}$ $yr^{-1}$; N3, 240 kg N $ha^{-1}$ $yr^{-1}$.

3.30 ± 0.73 cm, 3.51 ± 0.73 cm, and 2.72 ± 0.65 cm for N0, N1, N2, and N3, respectively. There were no statistical differences in soil temperatures and water table depth among different treatments (Table 2). With regard to interannual variations, no significant differences in soil temperatures were observed for N0, N1, N2, and N3, respectively ($P > 0.05$, Table 3), while year exerted an effect on water table depth ($P < 0.05$, Table 3). The aboveground biomass showed the highest value in N3 treatment ($P < 0.05$), while there were no significant differences in the other three treatments ($P > 0.05$, Table 2). However, there was a significant interaction between N addition level and year on aboveground biomass ($P < 0.001$, Table 3). Over 2005 and 2007, aboveground biomass in N3 treatment showed higher values than N0, N1, and N2 treatments ($P < 0.05$). No significant differences were observed among N1, N2, and N3 treatments, but they showed higher value than N0 treatment (Figure 2).

### 3.2. GHG Fluxes Under Various N Levels Following 5 Year N Fertilization

[15] The 5 year average ecosystem respiration in the N0 plot was 861.89 ± 39.88 mg C $m^{-2}$ $h^{-1}$, and they were 861.04 ± 41.00, 1027.33 ± 50.16, and 1244.31 ± 208.17 mg C $m^{-2}$ $h^{-1}$ in N1, N2, and N3 treatments, respectively (Figure 3). The ecosystem respiration observed in N1 treatment was insignificant compared to the control plot ($P > 0.05$), while ecosystem respiration in N2 and N3 treatment was significantly enhanced relative to the control plot ($P < 0.05$). Ecosystem respiration showed no significant differences between N1 and N2 treatments. However, the N3 plot increased the ecosystem respiration by 55% and 32%, respectively, compared to those in N1 and N2 plots ($P < 0.05$). Overall, there was no significant difference in $CH_4$ emissions among the N0,

**Table 5.** Correlation Coefficients Between Greenhouse Gases and Soil Temperatures at 5 cm Depth Under Different N Addition Levels[a]

| | $CO_2$ | | | | $CH_4$ | | | | $N_2O$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N0 | N1 | N2 | N3 | N0 | N1 | N2 | N3 | N0 | N1 | N2 | N3 |
| 2007 | 0.198 | 0.471 | 0.264 | 0.268 | −0.517 | −0.200 | −0.433 | −0.561* | 0.722** | 0.356 | 0.783** | 0.649* |
| 2008 | 0.207 | 0.515* | 0.667** | 0.406 | −0.72** | −0.672** | −0.727** | −0.661** | 0.281 | 0.631* | 0.379* | 0.543 |
| 2009 | −0.216 | 0.108 | 0.335 | −0.108 | 0.731* | 0.575 | 0.671 | 0.491 | 0.731* | −0.342 | −0.108 | −0.898** |
| 2007–2009 | 0.356** | 0.624** | 0.659** | 0.556** | −0.316** | −0.313** | −0.289** | −0.218 | 0.379** | 0.145 | 0.216 | 0.182 |

**Significance at $P < 0.01$.
*Significance at $P < 0.05$.
[a]There is no statistical significance for values without asterisks.

**Table 6.** Correlation Coefficients Between Greenhouse Gases and Water Table Depths Under Different N Addition Levels[a]

| | $CO_2$ | | | | $CH_4$ | | | | $N_2O$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N0 | N1 | N2 | N3 | N0 | N1 | N2 | N3 | N0 | N1 | N2 | N3 |
| 2007 | −0.762** | −0.87** | −0.844** | −0.704** | 0.353 | 0.389 | 0.509 | 0.276 | −0.474 | −0.294 | −0.567 | −0.506* |
| 2008 | −0.449 | −0.541* | −0.691** | −0.555* | 0.623* | 0.628* | 0.663* | 0.623** | −0.605* | −0.71** | −0.643** | −0.72** |
| 2009 | −0.027 | 0.7 | 0.156 | −0.109 | 0.436 | 0.518 | 0.464 | 0.245 | 0.317 | 0.19 | 0.191 | −0.3 |
| 2007–2009 | −0.493** | −0.607** | −0.721** | −0.488** | 0.479** | 0.547** | 0.594** | 0.476** | −0.515** | −0.538** | −0.545** | −0.64** |

**Significance at $P < 0.01$.
*Significance at $P < 0.05$.
[a]There is no statistical significance for values without asterisks.

N1, N2, and N3 treatments. On average from 2005 to 2009, the largest average $N_2O$ release occurred in N3 treatment ($0.52 \pm 0.09$ mg N $m^{-2}$ $h^{-1}$), and increased by 396%, as compared to $N_2O$ emission of $0.10 \pm 0.03$ g N $m^{-2}$ $h^{-1}$ in control plot ($P < 0.05$). However, no significant differences in $N_2O$ emissions were observed among the N0, N1, and N2 treatments ($P > 0.05$).

### 3.3. Interannual GHG Fluxes Under Various N Levels

[16] There was a significant interaction between N addition and year on ecosystem respiration, and also $N_2O$ emissions ($P < 0.05$, Table 4), whereas this interaction was not detected on $CH_4$ emissions ($P > 0.05$, Table 4). Compared to the control plot, ecosystem respiration in the N3 plot increased by 139% in 2005, while such significant difference did not occur in other years with continuous N enrichment ($P > 0.05$). The magnitude of increase in the N3 treatment was 98% and 67%, respectively, as compared to the N1 and N2 addition plots, in 2005. However, no significant differences in ecosystem respiration between the N3 plots and the other plots were observed in 2007 and 2009 ($P > 0.05$). In 2008, the N2 plot showed slightly higher ecosystem respiration values compared to those in the control, N1 and N3 plots ($P < 0.05$). However, no significant differences were observed in 2007 and 2009. It is notable that no significant differences in ecosystem respirations were observed between N0 and N1 treatment in 2005, 2007, 2008, and 2009 ($P > 0.05$).

[17] N addition had no effect on the $CH_4$ emissions among the four treatments over the growing season of 4 years ($P > 0.05$; Table 4 and Figure 4). For $N_2O$ flux, there were no significant differences among the control, N1, and N2 plots ($P > 0.05$). The $N_2O$ emissions in the N3 site increased by 576%, 631%, and 264%, respectively, compared to those from control, N1, and N2 plots in 2005 ($P < 0.05$) and 574%,

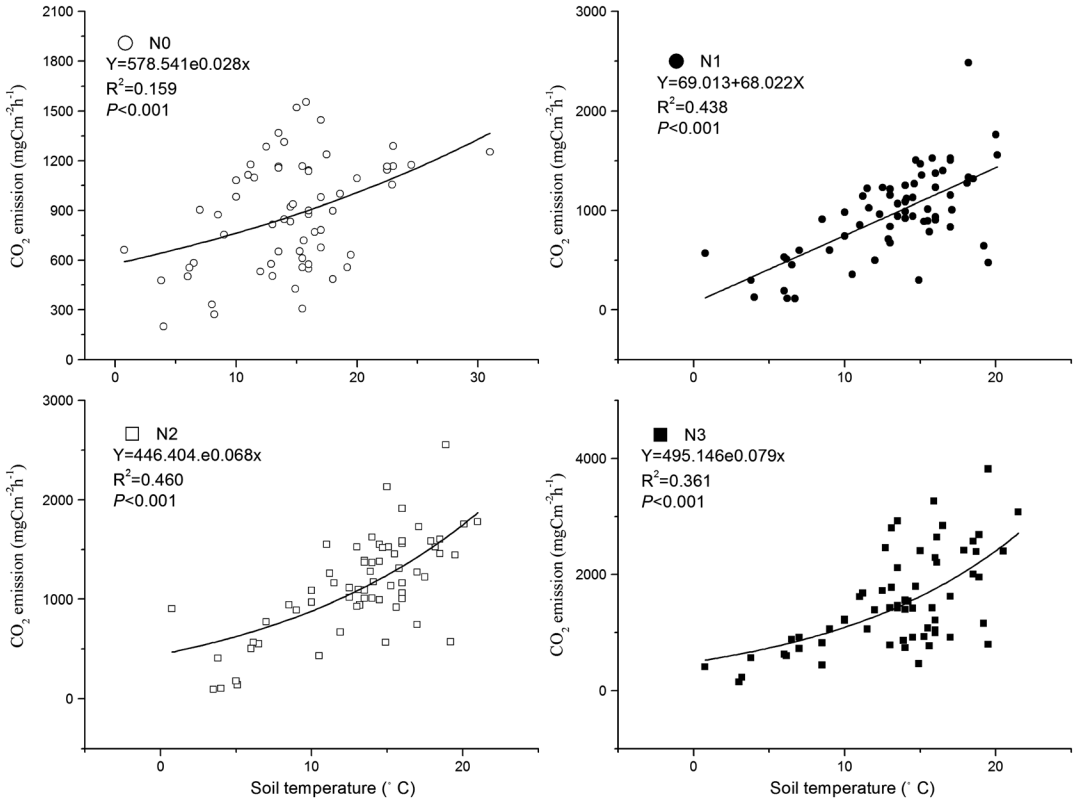


**Figure 5.** The relationship between $CO_2$ flux rate and soil temperature at 5 cm depth. N0, 0 kg N $ha^{-1}$ $yr^{-1}$; N1, 60 kg N $ha^{-1}$ $yr^{-1}$; N2, 120 kg N $ha^{-1}$ $yr^{-1}$; N3, 240 kg N $ha^{-1}$ $yr^{-1}$.

241%, and 223%, respectively, in 2009 ($P < 0.05$). However, there was no significant difference between N3 plot and the other sites in 2007 and 2008.

### 3.4. Dependence of GHG Emissions on Soil Temperature and Water Table Depth

[18] Across the four treatments, the relationship between GHG and soil temperature at 5 cm depth or water table depth varied among different N fertilization level and years (Tables 5 and 6). These correlations are complicated. Over the observation period from 2007 to 2009, however, we found that soil temperature at 5 cm depth showed significantly positive correlation with ecosystem respiration at N0, N1, N2, and N3 plots (Table 5). As for $CH_4$ flux, significantly negative correlations were observed between soil temperature at 5 cm depth and N0, N1, and N2 plots over 2007 to 2009, while there was no correlation between $CH_4$ flux and soil temperature at 5 cm depth for the N3 plot. There was significant correlation between $N_2O$ fluxes under N0 plot and soil temperature at 5 cm depth, but $N_2O$ fluxes under N1, N2, and N3 plots exerted no relationship with soil temperature at 5 cm depth ($P > 0.05$). Significantly negative correlation was found between water table depth and ecosystem respiration or $N_2O$ fluxes at all the sites, while $CH_4$ fluxes at the four plots showed positive correlation with water table depth (Table 6).

[19] As shown in Figure 5, we found that $CO_2$ flux rates exponentially increase with soil temperature at 5 cm depth at the N1, N2, and N3 plots ($P < 0.001$; $R^2 = 0.16$, 0.46, and 0.36, respectively) and linearly increase with soil temperature at the N1 plot ($P < 0.001$; $R^2 = 0.44$). $Q_{10}$ values were calculated by the exponential regression between $CO_2$ flux rate and soil temperature at 5 cm depth (Figure 5). We found that the temperature sensitivity of ecosystem respiration increased from control plot ($Q_{10} = 1.32$) to the N2 and N3 plots ($Q_{10} = 1.98$, 2.21) ($P < 0.05$). However, the $Q_{10}$ values at 5 cm showed no differences under the N2 and N3 plots ($P > 0.05$).

## 4. Discussion

### 4.1. Effects of N Addition on GHG Fluxes

#### 4.1.1. Ecosystem Respiration

[20] Our study found that 5 years of N fertilization increased ecosystem respiration only under the N3 plot with N addition at 240 kg N $ha^{-1}$ $yr^{-1}$ rate. In addition, the N3 plot exerted higher ecosystem respiration based on the average of 5 year observation compared to those in the N addition plots at 60 and 120 kg N $ha^{-1}$ $yr^{-1}$ rates. *Saarnio et al*. [2003] reported statistically insignificant changes in $CO_2$ exchanges under raised $NH_4NO_3$ supply at a rate of 30 kg N $ha^{-1}$ $yr^{-1}$ in boreal oligotrophic mire in eastern Finland. *Bragazza et al*. [2006] found that higher atmospheric N supplies resulted in higher $CO_2$ emissions in European bogs. In addition, *Keller et al*. [2005] demonstrated that there was no N addition effect on $CO_2$ emissions in an intermediate fen of northern Minnesota, with two N levels (20 or 60 kg N $ha^{-1}$ $yr^{-1}$), which is equal to or lower than our amounts of low-level N fertilization. As carbon and nitrogen dynamics differ under different N fertilization plots, it is possible that ecosystems respiration may respond differently to different rates of N fertilization. Thus, studies assessing the potential effect of N deposition on ecosystem respiration must carefully take N addition levels into consideration.

[21] As for time scale, our study shows that high-level N fertilization in the first year stimulated ecosystem respiration by 139%, but it had no effect in the following years. A similar temporal response to long-term N additions was observed for oak-dominated hardwoods at Harvard Forest, with soil respiration increase occurring in the first year of N additions (50 and 150 kg N $ha^{-1}$ $yr^{-1}$), followed by reductions in soil $CO_2$ efflux after 13 years of treatment [*Bowden et al*., 2004]. *Burton et al*. [2004] also reported that soil respiration rates were slightly higher in the $NO_3^-$-N amended plots during the first year, while they depressed by 15% in the eighth year of chronic $NO_3^-$-N additions in northern hardwood forests.

[22] Indeed, we also found that the middle N addition level held higher ecosystem respiration than the other N addition plots in the fourth year of N addition, while it showed no differences with control plot in the other years of N addition. Interestedly, the response of aboveground biomass to middle N addition level showed the same changing tendency as the ecosystem respiration did (Figure 2). It implied that vegetation dynamics in response to N fertilization might interact with the changes of ecosystem respiration [*Saarnio et al*., 2003]. *Zhang et al*. [2007b] reported that ecosystem respiration was significantly correlated with N content in plant, aboveground biomass, and plant height. Shoot respiration is vital in ecosystem respiration from wetland ecosystems [*Roehm*, 2005]. One study in this area demonstrated that the aboveground biomass contributed 50% to ecosystem respiration [*Lu*, 2008]. Also, fertilizer affecting plant growth and subsequent uptake of N and quality of litter [*Mao et al*., 2012] will significantly interact with fertilizer effects on $CO_2$ production.

#### 4.1.2. $CH_4$ Fluxes

[23] Generally, wetland soils are the main natural source with an estimated emission of 100–200 Tg $CH_4$ $year^{-1}$ [*Mer and Roger*, 2001]. N fertilizer is an important factor governing the $CH_4$ production, consumption, and emission from wetland soils [*Bodelier*, 2011]. Our study demonstrated that the effects of N addition on $CH_4$ emissions were insignificant. This agrees well with *Silvola et al*.'s [2003] study, which reported that the N treatment resulted in nonsignificant differences in methane emissions at three sites representing oligotrophic peatlands along a European transect. *Liu and Greaver* [2009] executed a meta-analysis including 109 studies covering North and South America, Europe, and Asia and reported that $CH_4$ emission increased by 95% averaged over all ecosystem types with N enrichment from 30 to 400 kg N $ha^{-1}$ $year^{-1}$. Another meta-analysis covering 33 published papers that contained $CH_4$ emissions observations from N fertilizer (28–406 kg N $ha^{-1}$) treatment and its control have indicated that N fertilizers increased $CH_4$ emissions in 98 of 155 data pairs in rice soils [*Banger et al*., 2012]. *Dong et al*. [2011] and *Xie et al*. [2010] found N addition inhibited $CH_4$ emissions in rice field.

[24] The effects of N addition on $CH_4$ emissions in our study and other studies were complicated, as the effects of N deposition on ecosystem $CH_4$ dynamics are complicated and may take place at biochemical, microbial, and ecosystem levels [*Schimel*, 2000]. These processes are mediated by microbes such as methanogens and methanotrophs [*Keller et al*., 2005]. It has been reported that moderate N

addition could stimulate the activities of methanogens, while excessive N input could be toxic to methanogens and thus inhibit $CH_4$ production [*Reay and Nedwell*, 2004; *Xu and Inubushi*, 2004]. N fertilization has also been shown to suppress the activity of methanotrophic bacteria, resulting in different effects depending on the dose of N addition [*Bodelier and Laanbroek*, 2004; *King and Schnell*, 1998]. *Nykanen et al*. [2002] found that no difference in $CH_4$ production or consumption potentials in a Sphagnum fuscum-dominated peatland was observed following N fertilization, though they did find an increase in $CH_4$ emissions. This might be because other factors could also have contributed to or interacted with $CH_4$ dynamics affected by N addition. For example, *Keller et al*. [2005] reported that N fertilization appeared to result in a shift in the dominant plant community structure. *Nykanen et al*. [2002] found that N addition influenced root exudates and then $CH_4$ production. Also, interactions between the nitrogen and $CH_4$ cycle are complex and far from understood [*Bodelier*, 2011]. More parameters, such as plant community structure, plant litter, root exudates, and roots [*Banger et al*., 2012], or interactive functions between microbes and plants [*Bodelier*, 2011], ought to be further investigated in the future to thoroughly explain the N-$CH_4$ dynamics.

#### 4.1.3. $N_2O$ Fluxes

[25] In our study, $N_2O$ emissions were increased by an average of 396% under high-level N addition (240 kg N $ha^{-1}$ $year^{-1}$). However, it is notable that the low and medium-N addition (60 and 120 kg N $ha^{-1}$ $year^{-1}$) showed no significant differences in $N_2O$ emissions compared to the control plot during the 5 year N addition experiment ($P > 0.05$), while $N_2O$ emissions in the high-level N fertilization plot was significantly higher than those in the low and medium-level N addition plots. *White and Reddy* [1999] also found that significant increases in denitrification rates in the Everglade soils were more pronounced in response to high N treatments than low N treatments, though *Nadelhoffer et al*. [1999] reported that soil $N_2O$ emission exhibited positively linear relationships with the rates of N inputs in nine temperate forests in Europe. *Liu and Greaver* [2009] concluded that $N_2O$ emission was significantly lower at low N addition rate (<55 kg N $ha^{-1}$ $year^{-1}$) compared with high N addition rate (>150 kg N $ha^{-1}$ $year^{-1}$), which agreed with our study.

[26] Unlike the case with ecosystem respiration, it is notable that $N_2O$ emission from the fifth year of high N addition showed more pronounced responses to N addition compared with that from the initial 4 years. That may be because a large proportion of fertilized N is retained in plants and surface soil organic matter over a shorter time period (<1 year), instead of being involved in nitrification or denitrification [*Curtis et al*., 2006], and thus have less effect on $N_2O$ fluxes.

#### 4.1.4. Environmental Controls on the Gas Fluxes

[27] The strong correlation between ecosystem respiration and soil temperature in the current study agrees well with other field studies conducted in wetland ecosystems [*Lafleur et al*., 2005; *Phillips et al*., 2010]. Ecosystem respiration increased with an increase in soil temperature [*Song et al*., 2009; *Phillips et al*., 2010], and this positive effect was clearly evident in this study as well. The $Q_{10}$ is commonly used to express the relationship between respiration rate and temperature [*Kirschbaum*, 2006]. The $Q_{10}$ for the respiration rate in the control treatment was 1.32. We found that the temperature sensitivity of ecosystem respiration increased for the medium and high-level N plots ($Q_{10} = 1.98$; 2.21) compared to that of control. This implies that the sensitivity of ecosystem respiration to soil temperatures would increase as the level of N input increases. Another study conducted in a temperate semiarid steppe also showed a greater temperature sensitivity of ecosystem respiration under N addition [*Yan et al*., 2011]. The enhanced temperature sensitivity of ecosystem respiration might be due to N stimulation of microbial activities under N addition.

[28] In our study, there was significantly negative relationship between variations of $CH_4$ fluxes and soil temperatures at the control, low, and medium-level N addition plots. However, we found that $CH_4$ flux was uncorrelated with soil temperature at 5 cm depth at the high-level N addition plot. Also, there were no correlations between $N_2O$ flux and soil temperature at 5 cm depth in N addition plots, though significantly positive relationship was observed between $N_2O$ flux and soil temperature at 5 cm depth for control plot. N addition might interact with the temperature-controlled $CH_4$ or $N_2O$ production processes, such that responses of related microbes [*Wrage et al*., 2001; *Banger et al*., 2012] to temperature change varied with occurrence of changing N availabilities. Significantly positive relationship was observed between $CH_4$ fluxes and water table depth in the current study, and this relationship was also found in other studies [*Updegraff et al*., 2001; *Huissteden et al*., 2006]. Ecosystem respiration and $N_2O$ fluxes showed negative relationship with water table depth in this study, which agrees well with the studies of *Chimner and Cooper* [2003] and *Huissteden et al*. [2006]. We hypothesize that responses of GHGs fluxes to N addition in wetland ecosystems might be interactively influenced by soil temperature, water table depth, and amount of fertilizer N.

[29] However, the relationships between soil microclimate conditions and GHGs on year scale are complicated (Tables 5 and 6). For example, the significant relationship between water table depth and $CH_4$ fluxes did not exist every year. Thus, it is hard to only contribute the interannual GHG dynamics to variations in the soil microclimate conditions. More detailed parameters need to be investigated in the future to explore the interannual variations in GHG dynamics under continuous N enrichment.

## 5. Conclusions

[30] This study presents results of a 5 year field experiment for net exchanges of $CH_4$ and $N_2O$, and ecosystem respiration in response to N addition in freshwater marsh in the Sanjiang Plain, Northeast China. The results indicated that high-level N fertilization in the first year stimulated ecosystem respiration more than it did in the following years, while low-level N addition showed no effect on ecosystem respiration over the study period. No statistically significant differences in $CH_4$ fluxes were found among the four N treatments. $N_2O$ emission from the first and fifth years of high-level N addition showed more pronounced responses to N addition compared with that from the third and fourth years. $N_2O$ emission under the low and middle-level N addition showed no significant difference with that from nonfertilized plot. The responses of GHG fluxes to N addition depend on gas species, experimental time, and

amounts of N fertilization. The results of our study affirm the importance of long-term field studies with multiple levels of N addition for assessing the impact of chronic N addition on the three GHGs, indicating that the current biogeochemical models based on short-term and single N input level's experiment might not be sufficiently robust for long-term application.

[31] To the best of our knowledge, this study is among the first long-term field observations simultaneously covering multiple greenhouse gases and multiple N addition levels conducted on natural wetlands. Given that natural wetlands are often nitrogen-limited and have significant influence on GHG budget [*Post et al*., 1982; *Chapin et al*., 2002], the information provided in this study may merit future research.

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