Effects of the addition of nitrogen and sulfate on CH₄ and CO₂ emissions, soil, and pore water chemistry in a high marsh of the Min River estuary in southeastern China

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ABSTRACT

Exogenous nitrogen (N) and sulfate (SO_4^{2-}), resulting from human activity, can strongly influence the emission of CH_4 and CO_2 from soil ecosystems. Studies have reported the effects of N and SO_4^{2-} on CH_4 and CO_2 emissions from inland peatlands and paddies. However, very few studies have presented year-round data on the effects of the addition of N and SO_4^{2-} on CH_4 and CO_2 emissions in estuarine marshes. The effects of the addition of N and SO_4^{2-} on the emission of CH_4 and CO_2 were investigated in a *Cyperus malaccensis* marsh in the high tidal flat of the Min River estuary of southeastern China from September 2014 to August 2015. Dissolved NH_4Cl , KNO_3 , and K_2SO_4 were applied every month, in doses of $24 \text{ g N/SO}_4^{2-} \text{ m}^{-2} \cdot \text{yr}^{-1}$. The emission of CH_4 and CO_2 showed distinct monthly and seasonal variations. Compared with the control, the addition of NH_4Cl and $\text{NH}_4\text{NO}_3 + \text{K}_2\text{SO}_4$ showed increases in CH_4 fluxes ($p < 0.05$), while the effects of the addition of KNO_3 and K_2SO_4 on CH_4 were minor ($p > 0.05$). NH_4Cl had a positive impact on CO_2 emissions ($p < 0.01$), while the addition of KNO_3 , K_2SO_4 , and $\text{NH}_4\text{NO}_3 + \text{K}_2\text{SO}_4$ had minor positive impacts, compared to the control ($p > 0.05$). Correlation analysis found that soil sulfate concentration, nitrogen availability and enzyme activity were the dominant factors influencing CH_4 and CO_2 variation. Our findings suggest that CH_4 and CO_2 emissions were influenced more by ammonium than by nitrate. We propose that the suppressive effect of additional sulfate on CH_4 production is insignificant, due to which the inhibition may be overestimated in the estuarine brackish marsh.

Keywords CH_4 and CO_2 ; Nitrogen and sulfate addition; Tidal marshes; High tidal flat; Min River estuary

1. Introduction

The atmospheric concentrations of carbon dioxide (CO₂) and methane (CH₄), key active greenhouse gases (GHG), have increased to 391ppm and 1803ppb in 2011, respectively, exceeding pre-industrial levels by about 40% and 150%, respectively (IPCC, 2013). Human activities, such as fossil fuel combustion and the use of agricultural fertilizers, have substantially elevated the nitrogen (N) and sulfate (SO₄²⁻) deposition (Gauci et al., 2008; Gundale et al., 2014). Particularly in China, the deposition intensity of N and SO₄²⁻ has continued to increase, making it the third largest N and SO₄²⁻ deposition zone in the world (Saikawa et al., 2009; Liu et al., 2013). A considerable number of studies have demonstrated that N and SO₄²⁻ enrichment have several known effects on biochemical reactions in the soil (Zhang et al., 2007; Richards and Pallud, 2016). Moreover, it may promote the emission of GHG, further contributing to global warming (Estop-Aragonés et al., 2016).

Wetlands have the highest carbon (C) density in terrestrial ecosystems, thus playing an important role in the global C cycle (Kayranli et al., 2010). Exogenous N enrichment has been identified as a critical factor in C metabolic processes (Kotowska and Werner, 2013). A review of the effects of N enrichment on GHG indicated that the addition of N increased CH₄ emission by 97% and reduced CH₄ uptake by 38% across all ecosystems, although it had no significant effect on the net ecosystem CO₂ exchange in non-forest natural ecosystems (Liu and Greaver, 2009). Considerable research has been carried out in wetland ecosystems, especially rice paddies and peatlands (Silvola et al., 2003; Banger et al., 2012). However, the conclusions remain uncertain. For instance, Das and Adhya (2014) found that CH₄ fluxes increased by 31.9% after urea addition in tropical paddy fields, while Yao et al. (2012) indicated that urea fertilization reduced CH₄ emissions from rice paddies in the Yangtze River Delta. Silvola et al. (2003) measured the CH₄ fluxes at five sites representing oligotrophic peatlands along a European transect, which revealed that the effect of N addition on CH₄ emission was not significant at the Finnish, Dutch and Swedish sites, while they were significantly lower at the British site, compared to the control plots. There are only a few studies on the effects of N on ecosystem respiration (ER) in different types of wetlands, all of which have generated contradictory conclusions. Bragazza et al. (2006) found that an enhanced atmospheric N supply results in higher CO₂ emissions and dissolved organic carbon (DOC) release. Johnson et al. (2010) demonstrated that the deposition of ammonia (NH₃) has no effect on CO₂ efflux from *Calluna vulgaris* soil, whereas both, ammonium (NH₄⁺-N) and nitrate (NO₃⁻-N), reduce CO₂ fluxes. Moreover, the effect of NH₄⁺-N and NO₃⁻-N on CH₄ and CO₂ emissions from the soil has been the subject of considerable debate (Min et al., 2011; Moseman-Valtierra et al., 2011; Fang et al., 2014).

Dissimilatory sulfate reduction is the dominant terminal step in anaerobic organic substrate mineralization (Gauci et al., 2008). Studies on the effects of SO_4^{2-} enrichment on wetland CH_4 emissions, mainly focusing on rice paddies and peatlands, generally show that high SO_4^{2-} concentrations can assist sulfate-reducing bacteria (SRB) in outcompeting methanogens for energy source utilization, as a result of having a greater affinity for acetate, H_2 and other substrates, thus potentially inhibiting CH_4 production (Purdy et al., 2003). Gauci et al. (2008) also suggested that the addition of SO_4^{2-} to rice fields could reduce the release of root exudates, which are a key source of substrate for CH_4 production, thus putting the methanogens at a disadvantage compared to SRB. Sulfate addition in wetlands affects CO_2 emissions by influencing sulfate reduction, causing it to become the dominant anaerobic C mineralization pathway (Craft et al., 2009). However, only a few studies have investigated the effect of SO_4^{2-} on the emission of CH_4 and CO_2 in estuarine tidal marshes, which have high concentrations of SO_4^{2-} due to saltwater intrusion, the underlying mechanisms for which are unknown. Moreover, extracellular enzymes in soil can be found mainly in soil microorganisms, root exudates and from the decomposition of animal and plant residues, plays an important role in the turnover of organic matter (Min et al., 2011). However, little information is available about the response patterns of enzyme activity (especially hydrolase and oxidase activities) on CH_4 and CO_2 emissions, with the application of varying N and SO_4^{2-} in estuarine marshes.

Coastal estuarine marshes, located along the intersection between sea and land, can sequester C at higher rates compared to other ecosystems (Moseman-Valtierra et al., 2011). In recent years, the increasing intensity of human activities has caused the enrichment of N and SO_4^{2-} in near-shore waters, becoming a key factor in changing the biogeochemical cycle of these environments (Thang et al., 2013; Mou et al., 2014). However, little is known about the individual and combined effects of increased N and SO_4^{2-} on the CH_4 and CO_2 emissions in estuarine marshes. In order to predict the future concentration of atmospheric CH_4 and CO_2 , better estimates regarding the effects of the addition of N and SO_4^{2-} on estuarine ecosystems are necessary. In China, the natural coastal wetland area has been estimated at more than $6.69 \times 10^4 \text{ km}^2$ (Guan, 2012). According to a recent report, the subtropical Shanyutan estuarine tidal marsh in the Min River estuary has been receiving a large amount of N and SO_4^{2-} due to upstream and tidal action (Fujian Provincial Oceanic and Fishery Administration, 2016). Studies on the influence of high N and SO_4^{2-} loading on CH_4 and CO_2 emissions in natural subtropical estuarine tidal marshes can enhance the current GHG emissions inventory, especially for China, where little previous work has been carried out (Moseman-Valtierra et al., 2011; Mou et al., 2014). The goals of the present work were to: (1) quantify the individual and combined effects of the addition of N and SO_4^{2-} on CH_4 and CO_2 emissions; (2) determine the effects of the addition of N and SO_4^{2-}

on soil and pore water chemistry; and (3) investigate the factors responsible for changes in CH₄ and CO₂ emission induced by the addition of N and SO₄²⁻.

2. Materials and Methods

2.1. Site description

The field experiment was carried out in the western part of the Shanyutan wetland, which is the largest natural wetland in the Nature Reserve of the Min River Estuary (26°00'36"-26°03'42"N, 119°34'12"-119°40'40"E) in southeastern China (Fig. 1). The climate is dominated by a subtropical monsoon, with spring-dominant rainfall, as well as an annual mean temperature of 19.6°C and precipitation of 1350 mm (Tong et al., 2014). The average salinity of the tidal water was 4.2 ± 2.5 (Tong et al., 2014). The dominant type of soil was coastal saline soil (Mou et al., 2014). The main vegetation types include the native species *Cyperus malaccensis*, *Phragmites australis*, and the invasive species *Spartina alterniflora* (Tong et al., 2014).

Fig. 1. Location of the study area and sampling sites (star) in the Min River estuary.

The tide is a typical semidiurnal tide with a high tide range of approximately 2.5-6 m, which follows a spring-neap-spring tidal cycle (Luo et al., 2014). In order to minimize the impact of tides and avoid the chambers being flooded by tidal water (Fig. 1), the experimental blocks were located in the *C. malaccensis* marsh of the high tidal flat zone, which only floods during the spring tide periods (approximately 3% of the year; Zhang et al., 2015), while remaining completely exposed during low or neap tides. *C. malaccensis* is widely distributed throughout the estuaries of southeastern China. The maximum height of *C. malaccensis* is approximately 1.4 m, with the aboveground and belowground biomass being $1188 \pm 280 \text{ g} \cdot \text{m}^{-2}$ and $1906 \pm 340 \text{ g} \cdot \text{m}^{-2}$, respectively (Zhang et al., 2015).

2.2. Experimental design and treatments

Parallel to the embankment along the Shanyutan wetland, three experimental blocks were established on

the high tidal flat, at intervals of 3 m. The selected sites exhibited uniform characteristics in terms of vegetation, soil and hydrology. In each block, five plots (each with 1 m×1 m dimension) were randomly assigned one of the five treatments, with a total of 15 treatment plots. A 1 m buffer strip separated each plot. Exogenous N and SO_4^{2-} were applied to these experimental blocks from September 2014 to August 2015. The 5 treatments were characterized by 24 g N $\text{m}^{-2}\cdot\text{yr}^{-1}$ (NH_4Cl), 24 g N $\text{m}^{-2}\cdot\text{yr}^{-1}$ (KNO_3), 24 g SO_4^{2-} $\text{m}^{-2}\cdot\text{yr}^{-1}$ (K_2SO_4), 24 g N+24 g SO_4^{2-} $\text{m}^{-2}\cdot\text{yr}^{-1}$ ($\text{NH}_4\text{NO}_3:\text{K}_2\text{SO}_4=1:1$), and a control, to which nothing was added. The ambient wet depositions of N and SO_4^{2-} were measured to be approximately 4-5 g N $\text{m}^{-2}\cdot\text{yr}^{-1}$ and 7-8 g SO_4^{2-} $\text{m}^{-2}\cdot\text{yr}^{-1}$, respectively (Li et al., 2015). The doses of N and SO_4^{2-} applied in this study were 5- and 3-times higher than the natural deposition, respectively. Such large amounts of N and SO_4^{2-} were applied to evaluate GHG emissions due to N- and SO_4^{2-} -saturated conditions that may occur in the future (Zhang et al., 2007; Mou et al., 2014). N and SO_4^{2-} powders were dissolved in 600 mL of deionized water and evenly sprayed over the plots once a month for a total of 12 equal applications throughout the year. Control plots were watered with the same quantity (600 mL) of deionized water. In order to maximize the retention of the treatment solution in the experimental plots, irrigation was carried out after the ebb of the neap tide (Moseman-Valtierra et al., 2011; Mou et al., 2014). Furthermore, to minimize the disturbance to the measurement sites during sampling, a wooden access boardwalk was constructed adjacent to the experimental blocks.

2.3. Gas collection and measurement

An enclosed, dark static chamber (Nykänen et al., 2002; Hirota et al., 2004; Moseman-Valtierra et al., 2011; Tong et al., 2014; Mou et al., 2014) was used to measure the CH_4 and CO_2 emissions at each plot. The chambers consisted of two parts: a stainless-steel bottom collar (35 cm×35 cm×35 cm) and a polyvinyl chloride (PVC) top chamber (35 cm×35 cm×140 cm). The collar was permanently inserted into the sediment (plants were enclosed inside the chamber), with a 5-cm protrusion above the soil surface. The top chambers were equipped with battery-driven fans to ensure complete mixing of the internal air. Moreover, they were covered with insulated cotton quilts to prevent an increase in the air temperature within the chambers, especially during summer. CH_4 and CO_2 measurements were made at 8:00-10:00 am during one day, with low or neap tide in each month. Gas samples were collected using 100 mL polypropylene syringes, equipped with three-way stopcocks, and stored in 100 mL gas sampling bags (Dalian Delin gas Packing Co., Ltd., China). A total of four gas samples were collected at 0, 10, 20, and 30 min after closure.

CH_4 and CO_2 concentrations were determined within 48 h of sampling, using a gas chromatograph (GC-2010, Shimadzu, Kyoto, Japan) equipped with a methane reformer and a flame ionization detector (FID).

CH₄ and CO₂ fluxes in the chamber were determined via regressions of concentration and time, with data being rejected if the R^2 values were ≤ 0.90 (Hirota et al., 2004).

2.4. Pore water collection and analysis

Pore water was sampled using *in situ* dialysis (Ding et al., 2004; Hu et al., 2016). Before September 2014, a series of pore water collection tubes (5 cm inner diameter), with sampling depths of 0-10, 10-20, and 20-30 cm, were installed in each chamber, leaving a 5-cm protrusion above the soil surface. The top of each tube was sealed tightly with a cover. Two days before the gas sampling dates, all of the pore water was evacuated from each collection tube. At the time of the gas sampling, pore water was withdrawn using 100 mL airtight glass syringes connected to a rubber hose, through which it was immediately transferred to different containers (Ding et al., 2004). This was carried out once a month with a total of 12 samples being taken throughout the year. Pore water samples were transported to the laboratory within 6 h and stored at 4°C for a week. For analysis, the pore water samples were filtered through a 0.22 μm filter (BiotransTM nylon membranes) and subsequently used to determine the concentrations of SO₄²⁻, Cl⁻, NH₄⁺-N, NO₃⁻-N and DOC. The SO₄²⁻ and Cl⁻ concentrations in the pore water were measured by an ion chromatograph (Dionex 2100, American), while the NH₄⁺-N and NO₃⁻-N concentrations were analyzed using flow injection analysis (Skalar Analytical SAN⁺⁺, Netherlands) and the DOC was determined by a SHIMADZU TOC-VCPH/CPN analyzer (fitted with an NPOC unit).

2.5. Soil collection and analysis

Soil cores (10 cm diameter) were collected *in situ* at depths of 0-10, 10-20, and 20-30 cm, during gas sampling in October 2014 (autumn), January 2015 (winter), April 2015 (spring) and July 2015 (summer), and stored at 4°C in the dark with their structures intact. After they were naturally dried and sieved, the soil pH, NH₄⁺-N, and NO₃⁻-N concentrations were measured. The soil pH was measured using an IQ150 instrument (IQ Scientific Instruments, USA). Soil NH₄⁺-N and NO₃⁻-N concentrations were analyzed using flow injection analysis (Skalar Analytical SAN⁺⁺, Netherlands), following a 2 mol·L⁻¹ KCl extraction. At the end of the experiment, the activities of extracellular enzymes in surface soil (0-10 cm) were determined. Soil hydrolase activity, including β -glucosidase (βG), cellobiohydrolase (CBH), *N*-acetylglucosaminidase (NAG) and acid phosphatase (AP), as well as oxidase activity—including Phenol Oxidase (PhOx) and Peroxidase (Perox) — were measured using a multifunctional microplate reader (Synergy H4, American), following the methods of Sinsabaugh et al. (2002). Moreover, on each sampling date, the soil temperature and electrical conductivity (EC) were measured *in situ* from each chamber (5, 10, and 15 cm). The temperature was measured using an IQ150 instrument (IQ Scientific Instruments, USA), while the soil conductivity was

measured using a 2265FS EC meter (Spectrum Technologies Inc., USA). At the end of the experiment, the density and height of the living branches of *C. malaccensis* were measured from every plot. In order to maintain the integrity of the plots, above- and below-ground biomass was not measured while the experiment was in progress. In addition, air temperature and rainfall data were collected by an automatic weather station (LSI-LASTEM, Italy) at 30-min time intervals.

2.6. Statistical Analysis

The results were presented as the mean with standard error. Differences in the gas flux from the fertilizer and control treatments were tested using one-way analysis of variance (ANOVA). The interaction of treatment×month used repeated measures ANOVA. Pearson correlation coefficients were performed to examine the relationship between the fluxes and individual environmental variables. A stepwise regression analysis was performed to screen the most influential environmental factors (soil and pore water) for soil CH₄ and CO₂ emissions in estuarine marshes. Statistical analyses were carried out using SPSS 19.0 and Microsoft Excel. The figures were drawn using Origin 7.5. Contour diagrams were plotted using Surfer 8.0 (Golden Software Inc., CO, USA), with Kriging interpolation and extrapolation. The effects on CH₄ and CO₂ fluxes due to the application of N and SO₄²⁻ were calculated following the methods of Yao et al. (2012). Effects (%) = (VN-VC)/VC*100%; where VN and VC are the CH₄ and CO₂ fluxes in the soil applied with N or SO₄²⁻, and control ones, respectively; a positive value indicates the promotion of CH₄ and CO₂ fluxes by N or SO₄²⁻ application, while a negative value indicates inhibitory effect. Statistical results with $p < 0.05$ were considered significant.

Cumulative gas emissions during the experimental period were calculated following the method of Menéndez et al. (2006), by multiplying the mean flux of two successive determinations by the length of the period between the measurements and adding the amount thus obtained, to the previous cumulative total. The Global Warming Potential (GWP) of CH₄ cumulative emissions on a 100-year scale was estimated using the following equation (Neubauer and Megonigal, 2015): $F = SGWP \times EF_{CO_2} + SGWP \times EF_{CH_4}$; where F is the CO₂-equivalent flux (kg CO₂eq ha⁻¹); $SGWP$ is the sustained-flux global warming potential for gas emissions, $SGWP$ values of CO₂ and CH₄ are 1 and 45 on a 100-year scale, respectively; EF_{CO_2} and EF_{CH_4} are the cumulative CO₂ and CH₄ effluxes (kg·ha⁻¹), respectively.

3. Results

3.1. Weather and vegetation

Air temperature and rainfall varied substantially and showed clear seasonal patterns over the one-year

period (Fig. 2a). Monthly average air temperature was relatively low in December and January, while it was higher in June and July. Monthly total rainfall reached the maximum in July and minimum in January 2015. The addition of N and SO_4^{2-} consistently increased the density of vegetation ($p>0.05$), while NH_4Cl , KNO_3 , and $\text{NH}_4\text{NO}_3+\text{K}_2\text{SO}_4$ had a slightly positive impact on the height of vegetation towards the end of the experiment ($p>0.05$; Fig. 2b).

Fig. 2. (a) Variations of monthly average air temperature and total rainfall from September 2014 to August 2015; and (b) changes in plant density and height at the end of the experiment. Values of plant density and height represent the means of three replicates; while the vertical lines indicate standard errors.

3.2. Soil characteristics

Significant seasonal changes were observed in soil EC (Fig. 3a), which peaked in December 2014 and reached a minimum in June and July 2015. Soil EC displayed a gradual decrease with depth during September 2014 to February 2015, while it increased with depth from March to August 2015. ANOVA showed that the addition of $\text{NH}_4\text{NO}_3+\text{K}_2\text{SO}_4$ increased the soil EC ($p<0.05$), while no effect was observed due to the addition of NH_4Cl , KNO_3 , and K_2SO_4 , compared to the control ($p>0.05$). The soil surface temperature followed similar seasonal patterns, with the maximum occurring in July 2015 and the minimum in December 2014 (Fig. 3b). There was no difference in soil temperature with the variation in depth ($p>0.05$).

Fig. 3. Monthly variations of soil EC (a) and temperature (b) at depths of 5, 10 and 15 cm under different treatments in the tidal marsh of the Min River estuary. Values represent the means of three replicates, while the vertical lines indicate standard errors. EC: electrical conductivity.

The addition of N and SO_4^{2-} tended to decrease the soil pH (Fig. 4a), although there was little difference when compared to the control ($p>0.05$). The NH_4^+ -N content was the highest in the 0-10cm layer for all of the soils, which was increased by 244% and 137% due to the addition of NH_4Cl and $\text{NH}_4\text{NO}_3+\text{K}_2\text{SO}_4$ ($p<0.01$) as compared to the control, respectively (Fig. 4b). However, the differences between the soils treated with KNO_3 , K_2SO_4 , and the control were not significant ($p>0.05$). The NO_3^- -N content in soil was higher with the addition of KNO_3 and $\text{NH}_4\text{NO}_3+\text{K}_2\text{SO}_4$ than in the control by 381% and 205% ($p<0.01$), respectively, while no change was observed for NH_4Cl and K_2SO_4 treatments ($p>0.05$; Fig. 4c). The NH_4^+ -N and NO_3^- -N contents from different treatments showed a similar seasonal pattern, which was, highest in autumn and lowest in spring (Fig. 4b, c).

Fig. 4. Seasonal variations in soil pH (a), $\text{NH}_4^+\text{-N}$ (b), $\text{NO}_3^-\text{-N}$ (c) content at depths of 0-10, 10-20 and 20-30 cm under different treatments in the tidal marsh of the Min River estuary. The various uppercase and lowercase letters on the bars indicate significant differences at the $p<0.05$ level due to the varying seasons and treatments, respectively. Values represent the means of three replicates, while the vertical lines indicate standard errors. $\text{NH}_4^+\text{-N}$: ammonium nitrogen; $\text{NO}_3^-\text{-N}$: nitrate nitrogen.

Compared with the control, the addition of NH_4Cl and $\text{NH}_4\text{NO}_3+\text{K}_2\text{SO}_4$ increased the βG and CBH activities ($p<0.05$) by 125%, 90% and 217%, 151%, respectively (Fig. 5). The addition of NH_4Cl and KNO_3 had a negative impact on NAG ($p<0.05$), which caused a 69% and 65% decrease, respectively, compared to the control. The addition of NH_4Cl , K_2SO_4 , and $\text{NH}_4\text{NO}_3+\text{K}_2\text{SO}_4$ stimulated the AP activity markedly ($p<0.01$). The addition of KNO_3 showed a highly increase in PhOx , compared to the control ($p<0.05$). On the other hand, the addition of NH_4Cl , K_2SO_4 , and $\text{NH}_4\text{NO}_3+\text{K}_2\text{SO}_4$ slightly inhibited the PhOx and Perox activities ($p>0.05$).

Fig. 5. Soil enzymatic activities under different treatments in surface soil (0-10 cm) in the tidal marsh of the Min River estuary at the end of the experiment. The various lowercase letters on the bars indicate significant differences at the $p < 0.05$ level among the different treatments. Values represent the means of three replicates, while the vertical lines indicate standard errors. β G: β -glucosidase; CBH: cellobiohydrolase; NAG: N-acetylglucosaminidase; AP: acid phosphatase; PhOx: Phenol Oxidase; Perox: Peroxidase.

3.3. Pore water parameter

NH_4^+ -N concentration in pore water was characterized by distinct seasonal variations, which decreased with soil depth for all treatments. It was higher with the addition of NH_4Cl and $\text{NH}_4\text{NO}_3 + \text{K}_2\text{SO}_4$, compared to the control ($p < 0.01$; Fig. 6). NO_3^- -N concentration increased by 423% and 300% compared to the control, due to the addition of KNO_3 and $\text{NH}_4\text{NO}_3 + \text{K}_2\text{SO}_4$ ($p < 0.01$). SO_4^{2-} concentrations from the addition of K_2SO_4 and $\text{NH}_4\text{NO}_3 + \text{K}_2\text{SO}_4$ were higher than the control ($p < 0.01$), while Cl^- concentrations were not different among the treatments ($p > 0.05$). DOC concentrations in pore water across all treatments varied significantly across the sampling months, while it varied only slightly with depth (Fig. 6). DOC concentration was higher with the addition of NH_4Cl and $\text{NH}_4\text{NO}_3 + \text{K}_2\text{SO}_4$, compared to the control ($p < 0.01$).

Fig. 6. Monthly variations in the concentrations of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, SO_4^{2-} , Cl^- , and DOC in the pore water at depths of 0-10, 10-20 and 20-30 cm under different treatments in the tidal marsh of the Min River estuary. Values represent the means of three replicates. $\text{NH}_4^+\text{-N}$: ammonium nitrogen; $\text{NO}_3^-\text{-N}$: nitrate nitrogen; SO_4^{2-} : sulfate ion; Cl^- : chloride ion; DOC: dissolved organic carbon.

3.4. CH_4 emissions

The estuarine marsh acted as a net source of CH_4 over the course of the experiment. CH_4 emissions showed distinct seasonal variations, particularly from April to August 2015 (Fig. 7). All of the treatments showed similar seasonal patterns, with the CH_4 flux being the highest during the summer months and the lowest during winter. Compared with the control, CH_4 fluxes increased by 164% and 113% in the marsh sites with additional NH_4Cl and $\text{NH}_4\text{NO}_3+\text{K}_2\text{SO}_4$, respectively ($p<0.05$). However, although CH_4 fluxes in the marsh site with K_2SO_4 decreased by 28% and the CH_4 emission from the KNO_3 treatment site increased by 23%, there were no significant differences with the control ($p>0.05$).

Fig. 7. Effects of the addition of nitrogen and sulfate on CH_4 fluxes in the tidal marsh of the Min River estuary. The various uppercase and lowercase letters on the bars indicate significant differences at $p<0.05$ level among the different seasons and treatments, respectively. Values represent the means of three replicates, while the vertical lines indicate standard errors. Seasonal fluxes are calculated as the average of three months for each season.

3.5. CO_2 emissions

The addition of N and SO_4^{2-} increased the peak value of CO_2 , which was the highest in July 2015 and lowest in December 2014 (Fig. 8). The dramatic effect of the addition of N and SO_4^{2-} on CO_2 emissions began to appear from March 2015. Compared with the control, the addition of NH_4Cl increased average CO_2 fluxes by 158%, with a positive impact ($p<0.05$). Although the addition of KNO_3 , K_2SO_4 , and $\text{NH}_4\text{NO}_3+\text{K}_2\text{SO}_4$ increased CO_2 fluxes by 55%, 71%, and 101%, respectively, there was no difference compared with the control ($p>0.05$).

Fig. 8. Effects of the addition of nitrogen and sulfate on CO₂ fluxes in the tidal marsh of the Min River estuary. The different uppercase and lowercase letters on the bars indicate significant differences at the $p < 0.05$ level among the different seasons and treatments, respectively. Values represent the means of three replicates, while the vertical lines indicate standard errors. Seasonal fluxes are calculated as the average of three months for each season.

3.6. Relationships between CH₄ and CO₂ emissions and environmental variables

CO₂ and CH₄ emissions from all treatments were positively correlated to soil temperature and pore water DOC ($p < 0.05$ or $p < 0.01$), while being negatively correlated to soil EC and pore water Cl⁻ ($p < 0.01$; Table 1). CO₂ and CH₄ emissions were positively correlated to the soil NH₄⁺-N and pore water NH₄⁺-N from NH₄Cl and NH₄NO₃+K₂SO₄ treatments ($p < 0.05$). CO₂ emissions were positively correlated to the soil NO₃⁻-N and pore water NO₃⁻-N from KNO₃ and NH₄NO₃+K₂SO₄ treatments ($p < 0.05$). CH₄ emissions were negatively correlated to the pore water NO₃⁻-N from KNO₃ treatment ($p < 0.05$). Except for the NH₄Cl treatment, CO₂ and CH₄ emissions were negatively correlated to pore water SO₄²⁻ with all other treatments ($p < 0.05$ or $p < 0.01$).

Table 1 Pearson correlation coefficients for CH₄, CO₂ fluxes and environmental properties in the tidal marsh

Environment	Factors	CH ₄ fluxes					CO ₂ fluxes				
		CK	NH	NO	S	NS	CK	NH	NO	S	NS
Soil	ST	0.660**	0.381*	0.612**	0.559**	0.506**	0.868**	0.831**	0.857**	0.881**	0.879**
	EC	-0.616**	-0.316	-0.550**	-0.479**	-0.440**	-0.816**	-0.726**	-0.806**	-0.814**	-0.822**
	pH	-0.013	-0.170	0.304	-0.176	0.159	0.122	0.235	0.260	0.169	0.437
	NH ₄ ⁺ -N	0.098	0.690*	0.500	-0.141	0.579*	0.158	0.622*	0.427	-0.002	0.524*
	NO ₃ ⁻ -N	-0.068	0.422	-0.468	-0.133	0.552	0.354	0.338	0.630*	0.187	0.707*
Pore water	NH ₄ ⁺ -N	0.297	0.571*	0.274	0.386	0.595*	0.619*	0.602*	0.571	0.521	0.634*
	NO ₃ ⁻ -N	-0.303	0.166	-0.552*	-0.135	0.085	-0.211	0.125	0.572*	-0.263	0.522*
	SO ₄ ²⁻	-0.741**	-0.545	-0.784**	-0.667*	-0.638*	-0.821**	-0.804**	-0.825**	-0.808**	-0.829**
	Cl ⁻	-0.807**	-0.607*	-0.793**	-0.764**	-0.738**	-0.766**	-0.762**	-0.829**	-0.743**	-0.841**
	DOC	0.750**	0.661*	0.883**	0.737**	0.773**	0.766**	0.825**	0.853**	0.833**	0.877**

The symbols * and ** indicate significant correlations at the 0.05 and 0.01 levels, respectively. Values of soil and pore water were calculated as the means of different vertical depths. CK, NH, NO, S, and NS represent the treatments of control, NH₄Cl, KNO₃, K₂SO₄, and NH₄NO₃+K₂SO₄, respectively. ST: soil temperature; EC: electrical conductivity; NH₄⁺-N: ammonium nitrogen; NO₃⁻-N: nitrate nitrogen; SO₄²⁻: sulfate ion; Cl⁻: chloride ion; DOC: dissolved organic carbon.

Regression analysis showed that the CH₄ flux was significantly related to soil temperature, EC, NH₄⁺-N, pore water SO₄²⁻, and DOC in the estuarine marshes ($p < 0.001$), which accounted for 72.2% of CH₄ emissions. Meanwhile, CO₂ flux was mainly controlled by soil temperature and pore water SO₄²⁻, NO₃⁻-N and DOC ($p < 0.001$), which accounted for 87.6% of CO₂ emissions (Table 2).

Table 2 Regression equations between CH₄ and CO₂ emissions, and environmental factors in the Min River estuary

Gas fluxes	Regression equations	F	R ²	P
CH ₄	$Y = 4.102 + 0.146 X_{ST} - 0.182 X_{PSO_4^{2-}} - 0.213 X_{EC} + 0.049 X_{PDO} + 0.056 X_{SNH_4^+-N}$	39.303	0.722	<0.001
CO ₂	$Y = 1725.946 + 68.100 X_{ST} + 116.287 X_{PDO} - 553.314 X_{PSO_4^{2-}} + 406.905 X_{PNO_3^--N}$	105.392	0.876	<0.001

ST: soil temperature; EC: electrical conductivity; PDOC: pore water DOC; SNH₄⁺-N: soil NH₄⁺-N; PSO₄²⁻: pore water SO₄²⁻; PNO₃⁻-N: pore water NO₃⁻-N. Values of the pore water and the CH₄ and CO₂ emissions were calculated as the means of each season (n=60).

Correlation analysis showed that CH₄ emissions were positively correlated to βG and CBH ($p < 0.05$), while they were negatively correlated to NAG ($p < 0.05$; Table 3). In addition, CH₄ emissions were slightly positively correlated to oxidase enzymes ($p > 0.05$). CO₂ emissions from all treatments were positively correlated to CBH ($p < 0.01$) and negatively correlated to NAG ($p < 0.05$).

Table 3 Pearson correlation for CH₄, CO₂ emissions and soil enzymatic activities in the tidal marsh of the Min River estuary

Gas fluxes	Hydrolase				Oxidase	
	βG	CBH	NAG	AP	PhOx	Perox
CH ₄	0.682*	0.519*	-0.559*	0.350	0.137	0.319
CO ₂	0.494	0.741**	-0.521*	0.182	0.281	-0.372

The symbols * and ** indicate significant correlations at the 0.05 and 0.01 levels, respectively. βG: β-glucosidase; CBH: cellobiohydrolase; NAG: N-acetylglucosaminidase; AP: acid phosphatase; PhOx: Phenol Oxidase; Perox: Peroxidase.

4. Discussion

4.1. Temporal variation of CH₄ and CO₂ emissions

4.1.1. CH₄ emissions

In this study, CH₄ emissions from the estuarine marshes greatly varied in seasonal dynamics, reaching a peak in June and July 2015 (Fig. 7), which was similar to the trend of temperature (soil temperature in particular; Fig. 3). The addition of N and SO₄²⁻ did not significantly affect the CH₄ emissions initially (except for the NH₄Cl treatment), while a significant positive effect was more apparent from April 2015 through the remainder of the experiment. The reason for this could be that increased temperature stimulates the decomposition of organic matter, which can eventually supply the substrate for CH₄ production (Ding et al., 2004). Zogg et al. (1997) also reasoned that dominant microbial communities at higher temperatures have the ability to metabolize substrates that are not used by members of the microbial community at lower temperatures. Additionally, the growing season of the vascular plant in this site, *C. malaccensis*, began in April. Therefore, the increase in biomass, caused by the addition of N, supported the transport of CH₄ without having to pass through the aerobic layer of soils (Chen et al., 2015). Hydrological conditions could be another possible reason, since the rainy season in this area begins in April every year (Fig. 2), with the anaerobic environment in waterlogged soils potentially limiting CH₄ oxidation. The suppressive effects of SO₄²⁻ treatments on CH₄ emission decreased in summer (Fig. 7). In general, increased temperature in the summer may increase the reaction rates of dissimilatory sulfate reductions (Sawicka et al., 2012), resulting in lower SO₄²⁻ concentrations in pore water (Fig. 6), which then affects CH₄ emissions in two ways. Firstly, low

SO_4^{2-} concentrations allow methanogens to outcompete the SRB for substrates, thus potentially stimulating CH_4 production (Purdy et al., 2003). Secondly, during the anaerobic oxidation of methane (AOM) process, CH_4 is oxidized with SO_4^{2-} as the terminal electron acceptor ($\text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O}$; Knittel and Boetius, 2009). The low SO_4^{2-} concentrations in pore water might inhibit the AOM process under anoxic conditions, which inhibits CH_4 oxidation, further promoting CH_4 emission from the estuarine marsh during the summer months (Fig. 7).

4.1.2. CO_2 emission

Wetland CO_2 emissions occur mainly through root respiration, microbial respiration and soil organic matter decomposition (Liu and Greaver, 2009). These processes are sensitive to the availability of C and N. Over the duration of the experiment, CO_2 emissions exhibited typical seasonal changes that broadly followed changes in temperature in the estuarine marsh (Fig. 8). The highest CO_2 emissions were observed during the summer months, which is the most favorable period for the assimilation and transformation of exogenous nutrients by microorganisms. Similar patterns were observed by Bridgman et al. (1995), who found that temperature, water and nutrient availability played a primary role in controlling the CO_2 released from natural wetlands. Jiang (2010) also found that the addition of exogenous nutrients can stimulate microbial activity and the decomposition of organic matter under appropriate temperature and water conditions, potentially leading to stronger respiration and higher CO_2 emissions. Moreover, the dramatic influences of the addition of N and SO_4^{2-} on CO_2 emissions began in April 2015 (rather than in the beginning), indicating that the peak value lags the nutrient application. In this regard, Yang et al. (2005) showed that there is a time lag in the appearance of the effects of the addition of exogenous nutrients on CO_2 emissions, after which the biomass increases significantly, which in turn increases root respiration and CO_2 emission. Mou et al. (2014) also believed that the temporal dynamics of GHG emissions from the estuarine marsh were probably due to the temporally varying responses of plants, animals and microorganisms to nutrient addition.

Table 4 Summary of the repeated measures ANOVA results for the treatments, time, CH_4 , and CO_2 emissions in the tidal marsh of the Min River estuary

		<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>
CH_4	NH	1	7.315	41.177	0.003
	Month	11	1.188	16.125	<0.001
	NH×month	11	0.226	3.074	0.004
	NO	1	0.153	1.131	0.348
	Month	11	0.747	11.652	<0.001
	NO×month	11	0.110	1.712	0.102
	S	1	0.212	5.376	0.081
	Month	11	0.496	12.817	<0.001
	S×month	11	0.068	1.768	0.090
	NS	1	3.467	6.390	<0.001
	Month	11	1.571	4.262	0.002
	NS×month	11	0.472	1.280	<0.001
CO_2	NH	1	4.022E7	149.952	<0.001
	Month	11	1.179E7	105.032	<0.001

NH×month	11	3351749.566	29.855	<0.001
NO	1	4697710.194	52.671	0.104
Month	11	5342906.666	61.073	<0.001
NO×month	11	507252.226	5.798	0.091
S	1	8066062.489	136.376	0.065
Month	11	5693070.971	63.189	<0.001
S×month	11	595720.921	6.612	0.268
NS	1	1.587E7	677.450	0.059
Month	11	7543200.060	188.472	<0.001
NS×month	11	1399246.929	34.961	0.002

NH, NO, S, and NS represent the addition of NH_4Cl , KNO_3 , K_2SO_4 , and $\text{NH}_4\text{NO}_3+\text{K}_2\text{SO}_4$, respectively.

4.2. The key factors for CH_4 and CO_2 emissions in estuarine marsh

4.2.1. Nitrogen availability

In this study, the addition of NH_4Cl led to a high increase in CH_4 emissions relative to the control ($p<0.05$; Fig. 7), especially with regard to the interaction with time ($p<0.01$; Table 4). CH_4 emissions were positively correlated to $\text{NH}_4^+\text{-N}$ concentrations in soil and pore water due to NH_4Cl addition ($p<0.05$; Table 1), indicating that $\text{NH}_4^+\text{-N}$ is a crucial factor influencing CH_4 emissions from estuarine marsh. $\text{NH}_4^+\text{-N}$ affects CH_4 emission mainly by regulating the metabolic processes of CH_4 production and oxidation. The mechanisms of $\text{NH}_4^+\text{-N}$ enrichment on CH_4 oxidation are mainly microbial and chemical. Microbially, the inhibitory effects of NH_4^+ on CH_4 oxidation have been confirmed in a variety of soil types (Zhu et al., 2010). The reasons for this include not only the competitive inhibition of methane monooxygenase, ammonia-oxidizing bacteria (AOB) and methanotrophs caused by NH_4^+ , but also the noncompetitive inhibition by the hydroxylamine and nitrite (NO_2^-) produced by NH_4^+ oxidation (King and Schnell, 1998). Furthermore, methanotrophs and AOB have a similar gene structure, when the soil is rich in $\text{NH}_4^+\text{-N}$, methanotrophs will shift from using CH_4 substrate to using NH_3 (Dubey, 2003). Regarding the chemical mechanism, Kravchenko (2002) suggested that the inhibitory effects of NH_4^+ on CH_4 oxidation can be partially explained by nonspecific ionic or salt effects. Physiological salt stress and the ion exchange of soil ammonium are caused by the addition of cations, which may lead to a decline in CH_4 oxidation (Kravchenko, 2002). The toxicity of NO_2^- and NO_3^- , generated by methanotrophic ammonia oxidation, is another possible explanation (Jiang et al. 2010). Additionally, $\text{NH}_4^+\text{-N}$ is the main source of N for methanogens in anaerobic conditions. Higher $\text{NH}_4^+\text{-N}$ can provide sufficient N substrates to enhance the abundance and activity of methanogens, contributing to CH_4 production (Kuzyakov and Xu, 2013). In this study, the high tidal flat in the Min River estuary was often N-deficit. Therefore, the addition of exogenous $\text{NH}_4^+\text{-N}$ resulted in higher CH_4 emissions by removing the N constraints. Additionally, the concentration of $\text{NH}_4^+\text{-N}$ was higher in the surface soil and pore water (Fig. 4; Fig. 6). Therefore, its promotion might be the highest in the surface layers of the tidal soil.

An increase in CH_4 emissions due to the addition of $\text{NH}_4\text{NO}_3+\text{K}_2\text{SO}_4$ was found in this study (Fig. 7),

which is in opposition to the finding by Eriksson et al. (2010). This may imply that the effect of the increase in plant biomass and microbial activity, caused by the addition of N on CH₄ production counteracted or weakened sulfate inhibition, especially in the estuarine marsh with low N and high SO₄²⁻ concentrations. Furthermore, apart from the effect of tides and saltwater intrusion, soil iron enrichment was also significant in the Min River estuary (Luo et al., 2014). The addition of exogenous N and SO₄²⁻ had a combined effect on CH₄ production, together with the electron acceptors SO₄²⁻ and Fe³⁺. For example, Neubauer et al. (2005) reported that HS⁻ (or S²⁻) and Fe³⁺ reducers can outcompete methanogens for substrates.

Correlation analysis showed that NO₃⁻-N concentration in pore water due to the addition of KNO₃, was negatively correlated with CH₄ fluxes ($p < 0.05$; Table 1), indicating that NO₃⁻-N inhibits the production and emission of CH₄ from estuarine marshes. On the one hand, the process of NO₃⁻-N denitrification is more favorable for substrate utilization than CH₄ production in an estuarine marsh, as it reduces the concentration of substrates for CH₄ production, due to which it cannot be utilized by the methanogens (Min et al., 2011). On the other hand, NO₃⁻-N treatment increases the NO₃⁻ concentration in soil and the toxicity of NO₂⁻ produced by the nitrification-denitrification processes, which may also suppress CH₄ production (Liu and Greaver, 2009). Nykänen et al. (2002) also indicated that NO₃⁻-N inhibits methanogenesis, as the microorganisms can use NO₃⁻ as electron acceptors to oxidize organic substrates (such as acetate) in the anaerobic environment, thus ensuring that methanogens cannot compete for substrates with nitrate reducers. However, an interesting outcome was that, although the addition of KNO₃ inhibited CH₄ emissions in field experiments over a few months, no differences were found ($p > 0.05$; Fig.7), suggesting that the addition of small pulses of exogenous NO₃⁻-N is not sufficient to significantly alter the CH₄ emission pattern in a short time. Most probably, the consumption of NO₃⁻ by denitrification can be the reason for the absence of any significant effects of the addition of NO₃⁻-N to this reduced environment. Moreover, NO₃⁻-N may also have been immobilized by microorganisms or assimilated by vascular plants in the upper aerobic soil layer, before it reached the anaerobic methanogenic zone (Nykänen et al., 2002). This was also confirmed by the characteristics of the surface enrichment of NO₃⁻-N in soil and pore water in this study (Fig. 4; Fig. 6).

ANOVA showed that the addition of NH₄Cl had a positive impact on CO₂ emissions ($p < 0.05$), while KNO₃ had a slightly more positive impact than the control ($p > 0.05$). This finding is consistent with the results presented by Currey et al. (2011) and Kivimäki et al. (2013), who found that the addition of NH₄⁺-N typically enhances soil DOC content, causing fertilized plots to become net sources of C to the atmosphere. With respect to NO₃⁻-N, less energy is required for microorganisms to absorb and utilize NH₄⁺-N, in turn promoting microbial activity and increasing the decomposition rate (Min et al., 2011). Consequently, we can

primarily draw the conclusion that ammonium is more effective than nitrate in increasing CO₂ emissions in estuarine marshes.

4.2.2. Sulfate concentration

In the present study, SO₄²⁻ concentrations in pore water were negatively correlated to CH₄ fluxes ($p < 0.05$ or $p < 0.01$), except for the NH₄Cl treatment (Table 1). It is generally acknowledged that coastal marshes have high concentrations of SO₄²⁻, which can help SRB to outcompete methanogens for energy sources due to its a greater affinity for acetate, H₂, and other substrates, eventually inhibiting CH₄ production (Purdy et al., 2003). However, an interesting and unexpected outcome of this study was that, there were no differences between CH₄ emissions from the addition of K₂SO₄ and the control ($p > 0.05$; Fig. 7). The mean SO₄²⁻ concentration in the pore water in the high marsh in Min River estuary was 4740 µmol·L⁻¹ (Fig. 6). This value is well above the SO₄²⁻ saturation concentrations of 300-3000 µmol·L⁻¹, reported for SRB (Vile et al., 2003), which suggests that the SO₄²⁻ concentration in the high marsh in Min River estuary was at saturation, with the effect of SO₄²⁻ on sulfate reduction being minor. The situation is different compared to studies on natural peatland and freshwater wetlands, where an increase in SO₄²⁻ availability in low SO₄²⁻ environments can significantly stimulate SRB, which then consume more substrate and inhibit methanogens (Gao et al., 2014). Vile et al. (2003) speculated that if methanogens and SRB dominate different vertical depths of the soil, with the SRB community showing more superficial distribution relative to the methanogens, the methanogens might not be exposed to exogenous SO₄²⁻, thus not being competitively inhibited. Furthermore, the diversity of methanogenic substrates present in estuarine marshes, such as noncompetitive substrates supplemented with methanol, trimethylamine or methionine, is another possible interpretation, as they are poorly used by SRB (Lyimo et al., 2002). Our results suggest that the suppressive effect of sulfate increases on CH₄ production may be overestimated in estuarine brackish marsh soil.

SO₄²⁻, as the terminal electron acceptor can affect organic matter mineralization and CO₂ emissions (Gauci et al., 2008). Chambers et al. (2011) found that the addition of SO₄²⁻ could enhance its significance as an inorganic electron acceptor, with the ratio of sulfate reduction and CO₂ production being 1:2. Unlike rice paddies and peatlands ecosystems, apart from the mineralization of organic carbon, CO₂ is also produced by the reduction of soil electron acceptors in estuarine marshes. Nevertheless, unlike the above-mentioned conclusions, an important implication of our study was that the CO₂ emission in each treatment was negatively correlated with the SO₄²⁻ in pore water ($p < 0.01$). Moreover, the addition of SO₄²⁻ did not cause a significant increase in CO₂ emissions (Fig. 8), without taking the effect of time into consideration (Table 4). This may have been due to the fact that the concentration of SO₄²⁻ in the Min River estuary was at saturation

(4740 $\mu\text{mol}\cdot\text{L}^{-1}$). The response may also have been delayed because the currently large SO_4^{2-} pools in these high tidal flats continue to provide SO_4^{2-} for sulfate reduction. It is also possible that SRB are affected by something other than SO_4^{2-} , even though SO_4^{2-} was abundant in the estuarine marshes. For example, a tentative interpretation is that, the principal pathway of CO_2 production in estuarine marsh is microbial decomposition of organic matter, a process heavily influenced by soil EC and pore water Cl^- . Soil EC, pore water Cl^- and SO_4^{2-} , are derived from seawater (Table 1), which can lead to the salt effect overpowering sulfate reduction, thereby inhibiting CO_2 production. A laboratory study also showed that a high concentration of Cl^- has a strong inhibitory effect on GHG emissions (Whalen, 2000), which was also confirmed by the negative correlation to pore water Cl^- in the study (Table 1). Another possible explanation is that the addition of SO_4^{2-} can, to some degree, result in soil acidification (Fig. 4), potentially influencing CO_2 production, although correlation analysis showed that pH was not a key factor in the control of CO_2 production (Table 1).

4.2.3. Soil enzyme activity

Soil hydrolytic enzymes mainly breakdown complex organic polymers into small organic molecules or monomers, subsequently splitting the glycosidic, ester and peptide bonds of organic compounds, thus significantly influencing the decomposition of organic matter (Sinsabaugh et al., 2002). Activities of βG , CBH and AP were greater in N and SO_4^{2-} treatments than the control ($p<0.05$; Fig. 5), most likely because the increased litter C and higher soil N availability could potentially stimulate microbial activity (Salome et al., 2010). In the present study, βG and CBH showed a positive correlation to CH_4 and CO_2 emissions ($p<0.05$; Fig. 5), indicating that hydrolytic enzyme activities are significant microbial factors regulating GHG emissions. The enhancement of the soil activity of hydrolytic enzymes can promote the decomposition and transformation of soil organic carbon, thus increasing the release of nutrients through enzymatic action (Taylor et al., 2002) and providing more substrate for the production of CH_4 and CO_2 . βG is an important hydrolytic enzyme involved in the release of C from organic matter. Hence, the enhancement of βG activity can increase the intensity of soil respiration and C turnover (Bragazza et al., 2006). A negative correlation was found between CH_4 and CO_2 emissions and NAG in this study ($p<0.05$). NAG was active when there was a demand for microbial N. The addition of N alleviated the microbial need for N sources, thereby decreasing NAG activity (Min et al., 2011). In addition, AP activity was mainly associated with the transformation and bioavailability of organic phosphorus (OP). OP can be hydrolyzed to inorganic phosphorus (IP), which can be absorbed and used by plants when AP activity is enhanced, thus providing more nutrients for plant growth (Bragazza et al., 2006). In this case, the positive relationship between the

addition of exogenous N and AP activity indicated that, with high N input, P availability plays a primary role in regulating litter and soil decay.

In general, the reduction in ligninase activity decreases the decomposition rate of litter that contains high lignin and increases the accumulation of soil organic C, ultimately promoting soil C sequestration (DeForest et al., 2004). Meanwhile, PhOx is an enzyme that can degrade polyphenols. When the decrease of PhOx activity and the degradation of lignin and other complex organic matter in soil is insufficient, the growth of heterotrophic microorganisms is inhibited (Sinsabaugh et al., 2002). As a result, C becomes the limiting nutrient of soil microbial communities (Andersson et al., 2004). In the present study, NO_3^- -N dominant treatment showed an increase in PhOx, relative to the control ($p < 0.05$; Fig. 5). This can reduce polyphenol concentrations during litter decay and stimulate the activity of hydrolase enzymes, eventually facilitating CH_4 and CO_2 emissions. However, no correlation was found between CH_4 and CO_2 emissions and oxidase activity ($p > 0.05$). This was most likely because, although oxidase activities are affected by N and SO_4^{2-} , CH_4 and CO_2 emissions are the result of many factors in estuarine marshes. Hence, slight changes in oxidase activity are not sufficient to affect CH_4 and CO_2 emission patterns. Currently, minimal research has been performed on the effect of oxidase activity on GHG emissions and further studies are essential.

4.3. Implications and future works

4.3.1. Implications

Cumulative gas emissions over a one-year period can help us update the gas inventory on a larger scale. Regarding the cumulative fluxes in this study (Table 5), the addition of NH_4Cl and $\text{NH}_4\text{NO}_3 + \text{K}_2\text{SO}_4$ increased cumulative CH_4 emissions ($p < 0.05$), while NH_4Cl addition increased the cumulative CO_2 emissions, relative to the control ($p < 0.05$). Low cumulative CH_4 emissions were measured in the treatments with K_2SO_4 , which were not statistically different from those in the control (Table 4). Our present results imply the potential of NH_4^+ -N addition in increasing cumulative CH_4 and CO_2 emissions in subtropical estuaries, which further enhances our understanding of the effect of N and SO_4^{2-} additions on CH_4 and CO_2 emissions on a one-year scale.

Global Warming Potential (GWP) has become an important way to evaluate the effect of the GHG emissions of an individual system on the radioactive forcing of the climate. Many studies have been conducted to improve the evaluation methods of GWP in various ecosystems (Frolking et al., 2006; Neubauer and Megonigal, 2015). Most notably, Neubauer and Megonigal (2015) have presented a sustained-flux global warming potential (SGWP, for gas emissions) and sustained-flux global cooling

potential (SGCP, for gas uptake) model, when gas fluxes persist over time. In the present study, we quantified and compared the radioactive forcing of CH₄ and CO₂ fluxes in the subtropical estuarine marsh, based on the SGWP values over a 100-year period (Table 5). The total CO₂-equivalent flux in the NH₄Cl treatments was 135.72×10³ kg CO₂eq ha⁻¹, which was approximately 2.6 times greater than the value of 51.23 ×10³ kg CO₂eq ha⁻¹ found for the control ($p<0.05$). We also calculated the GWP change for the whole year under N and SO₄²⁻ treatments to find that the contribution of CH₄ to carbon emissions was 1.52%, 2.09%, 1.51%, 0.71% and 1.99%, from the treatments of control, NH₄Cl, KNO₃, K₂SO₄ and NH₄NO₃+K₂SO₄, respectively. Our preliminary result indicates that the addition of NH₄Cl could lead to an increase in the global warming risk from the subtropical estuarine marsh. The contribution of CH₄ and CO₂ to regional carbon emissions has changed after the addition of N and SO₄²⁻.

Table 5 Cumulative CH₄ and CO₂ fluxes over one year experimental periods, and Global Warming Potential as affected by the addition of nitrogen and sulfate.

Treatment	CH ₄				CO ₂			
	Average (mg·m ⁻² ·h ⁻¹)	Cumulative fluxes (kg·ha ⁻¹ ·yr ⁻¹)	Effects (%)	CO ₂ eq (10 ³ kg·ha ⁻¹)	Average (mg·m ⁻² ·h ⁻¹)	Cumulative fluxes (10 ³ kg·ha ⁻¹ ·yr ⁻¹)	Effects (%)	Total CO ₂ eq (10 ³ kg·ha ⁻¹)
Control	0.20a	17.34a	-	0.78a	583.94a	50.45a	-	51.23a
NH ₄ Cl	0.73b	62.85b	262*	2.83b	1538.12b	132.89b	163*	135.72b
KNO ₃	0.30a	25.90a	49	1.17a	885.80a	76.53a	52	77.70a
K ₂ SO ₄	0.16a	13.82a	-20	0.62a	1004.54ab	86.79ab	72	87.41ab
NH ₄ NO ₃ +K ₂ SO ₄	0.50b	43.41b	150*	1.95b	1113.60ab	96.21ab	91	98.16ab

The symbol * indicate that the addition treatment significantly promotes CH₄ or CO₂ flux compared to the control at the 0.05 levels ($n=3$). The different lowercase letters within a column indicate significant differences among the different treatments at the $p<0.05$ level. The symbol - indicates that there are no data.

4.3.2. Uncertainties and future works

In the present study, the CH₄ and CO₂ emissions data for a brackish marsh in subtropical estuary induced by N and SO₄²⁻ addition, were first reported. Although this study was based on field measurements at the high tidal flat marsh, the results were still very important in assessing the future concentration of atmospheric CH₄ and CO₂ in the background of N and SO₄²⁻ enrichment in estuarine marshes. However, when the data are scaled up from local to regional-level, some uncertainties and future works must be considered carefully. First, hydrological conditions such as seasonal change in river water discharge and seawater intrusion across the estuarine tidal marshes might have a significant effect on CH₄ and CO₂ emissions, as well as pore water chemistry. In this site, although the *C. malaccensis* marsh of the high tidal flat zone only floods for approximately 3% of the year (Zhang et al., 2015), and remaining completely exposed during low or neap tides, hydrological conditions still exert an imperceptible influence on the GHG dynamics in this environment. For example, the subtropical climate is characterized by frequent heavy rains, resulting in rapid increase in rainfall and runoff in a short term. Moreover, the southeastern coast of China is also frequently affected by typhoons, resulting in severe saltwater intrusion. The biogeochemical cycle of

subtropical estuarine marshes was most likely affected by the alternation of saltwater and freshwater. Therefore, to precisely assess CH₄ and CO₂ emissions in the intertidal zone of the Min River estuary, hydrological conditions should be further investigated during the sampling period. Second, as this study only lasted one year, the results derived from this study only reflect the variations of CH₄ and CO₂ emissions following the N and SO₄²⁻ treatments for one year. Therefore, to get a clearer and more precise picture of the effects of N and SO₄²⁻ on CH₄ and CO₂ emissions, long-term observations and multi-frequency sampling under natural conditions should be carried out in the future. Finally, this study examined only CH₄ and CO₂ emissions, and basic environmental factors in field, while the contribution of CH₄ production and oxidation, microbial abundance and activity (methanogens, methanotrophs, and SRB), and methanogenic substrates under an exogenous application of N and SO₄²⁻ were lacking. These can be key factors which control the biogeochemical conditions, as well as the CH₄ and CO₂ emissions in estuarine tidal marshes. Thus, the driving mechanism behind this pattern needs to be investigated further.

5. Conclusions

CH₄ and CO₂ emissions from the high tidal flat marsh in the Min River estuary exhibited typical temporal dynamics, with the effects of exogenous N and SO₄²⁻ broadly following the changes over time. The addition of NH₄Cl showed increases in CH₄ and CO₂ emissions relative to the control ($p < 0.05$), while the effects of the addition of KNO₃ were minor ($p > 0.05$). This finding indicates that CH₄ and CO₂ emissions were mostly influenced by the addition of ammonium rather than nitrate. The effect of K₂SO₄ addition on CH₄ and CO₂ emissions was minor ($p > 0.05$). This result indicates that CH₄ emissions from a sulfate saturated estuarine tidal marsh did not respond positively to additional sulfate. Moreover, the addition of NH₄Cl, KNO₃ and K₂SO₄ increased the concentrations of NH₄⁺-N, NO₃⁻-N and SO₄²⁻ in soil and pore water ($p < 0.01$), respectively. Soil nitrogen and sulfate availability, as well as enzyme activity were the dominant factors influencing the CH₄ and CO₂ dynamics in an estuarine marsh.

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