Estuarine, Coastal and Shelf Science 243 (2020) 106878

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: http://www.elsevier.com/locate/ecss

Nitrogen input weakens the control of inundation frequency on soil organic carbon loss in a tidal salt marsh

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ARTICLE INFO

Keywords:
Tidal salt marsh
Nitrogen input
Inundation frequency
Soil CO_2 and CH_4 emissions
Soil DOC loss

ABSTRACT

The soil carbon (C) sequestration capacity of salt marshes is of considerable importance with respect to the mitigatiing the potentially detrimental consequences of global climate change. Given that tidal salt marshes are subjected to periodic cycles of inundation, it is assumed that soil C cycle in these marshes is expected to be controlled to varing extent by changes in soil moisture and salinity induced by the alternating patterns of drying and rewetting. In addition, with increases in the extent and severity of nitrogen (N) eutrophication becomes serious, we predicted that soil organic carbon (SOC) loss in tidal salt marshes is likely to be highly responsive to increased N loading. In this study, we conducted a two-factor mesocosm experiment (simulated inundation and N input in a tidal salt marsh) to determine the interactive effects of N eutrophication and inundation frequency on CO_2 and CH_4 emissions and dissolved organic carbon (DOC) contents. Our results showed that increased inundation frequency led to lower levels of CO_2 emission but greater CH_4 emission, whereas N input weakened the effects of changes in inundation frequency on CO_2 and CH_4 emissions. Moreover, N input was found to modify CO_2 and CH_4 emission in response to variations in soil moisture. We also observed an enhancement of soil DOC loss in response to increasing inundation frequency, and that DOC loss in the surface soil was considerably greater than that in subsurface soil. Further, as inundation frequency increased, we detected changes in the relationship between soil DOC and CO_2 and CH_4. Our findings highlighted that vertical variation in soil moisture induced by inundation is a key factor controlling SOC loss in tidal salt marshes, although N input can weaken this control.

1. Introduction

Despite the fact that tidal salt marshes cover obly a small fraction of the earth's surface, on account of their potentially considerable soil carbon (C) sequestration capacity (Duarte et al., 2013; McLeod et al., 2011). Given the abundant primary productivity of tidal salt marshes and their efficiency in trapping suspended particles, the rate of soil organic carbon (SOC) burial in these ecosystems is extremely high. Indeed, the carbon sequestration rate of tidal salt marshes has been estimated to be 40-fold higher than that of terrestrial forest soils (Duarte et al., 2013; McLeod et al., 2011). In addition, owing to the vertical accretion of sediments in healthy salt marsh ecosystems inresponse to rising sea levels, it has been predicted that the size of the “blue carbon” sink would constantly increase over time and never reach saturation point (Chmura et al., 2003; McLeod et al., 2011). However, tidal salt marshes can undergo a shift from being net C sinks to C sources, as a consequence of changes in natural (such as warming and exogenous nitrogen input) and anthropic (such as dam construction and reclamation) factors (IPCC, 2014; McLeod et al., 2011). Although vertical C losses, including emissions of CO_2 and CH_4, are generally considered to

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https://doi.org/10.1016/j.ecss.2020.106878
Received 21 February 2020; Received in revised form 15 May 2020; Accepted 4 June 2020

be main contributor to the loss of C in terrestrial ecosystems (Chen et al., 2018; Fang et al., 2017), in tidal salt marshes, lateral C loss, particularly that of dissolved organic carbon (DOC), should not be ignored, given the substantial lateral exchange of mass induced by periodical tidal activity (Bauer et al., 2013). Accordingly, it can be resoned that a better understanding of mechanisms of SOC loss in tidal salt marshes would make a potentially valuable contribution to improving "blue carbon" management against a backdrop of global climate change.

Owing to their strategic location as the transition between land and sea, tidal salt marshes can be periodically inundated, and the associated drying-rewetting cycles can become one of the key factors controlling soil C cycling in these marshes (Jia et al., 2017; Shi and Marschner, 2014). For example, water-saturated soilwould constitute an unfavorable medium for aerobic microorganisms and gases exchange, thus inhibiting the generaion and emission of CO_2 (Liu et al., 2017; Moyano et al., 2013). Conversely, post-drainage soil drying in coastal wetlands leads to an increase in soil heterotrophic respiration and a decline in CH_4 emission (Deppe et al., 2010; Malone et al., 2013). Furthermore, changes in salinity induced by the drying-rewetting process in tidal salt marshes may constitute a prominent stress factor for soil microbial populations (Landesman and Dighton, 2011), and in this regard, it has been found that the rate of SOC decomposition in tidal salt marshes decreases with increasing soil salinity (Liu et al., 2017; Qu et al., 2018). Moreover, drying-rewetting cycles could also influence the physical protection of organic matter by soil aggregates, thereby affecting the release of soil DOC in tidal salt marshes (Bauer et al., 2013). Currently, however, there is comparatively little information available regarding the influence of different inundation frequencies on DOC loss in tidal salt marshes.

The increasing extent and severity of eutrophication that in recent years has afflicted oceanic and coastal waters worldwide ()has led to an increase in nitrogen (N) loading, which is considered to be a critical factor affecting SOC loss from salt marshes (Breitburg et al., 2018; Zhang et al., 2010, 2016). Given the various differences in N input level, N form, initial soil properties and other parameters, the observed effects of N input on SOC loss tend to be inconsistent and disputed (Hu et al., 2019; Liu et al., 2018; Luo et al., 2019; Min et al., 2011; Zeng et al., 2018). For example, according to the stoichiometric decomposition theory, enhanced N availability induced by N input invariably stimulates SOC decomposition, due to a reduction in the energy expended by microbes in acquiring N (Melillo et al., 1982). However, the microbial N mining theory mantains that soil microbes acquire N through mineralizing SOC, and consequently, the rate of SOC mineralisation would decrease in response to an increase in N availability (Hartley et al., 2010). In general, it has been observed that SOC decomposition is stimulated at low N input level but suppressed at high N input levels (Hasselquist et al., 2012; Xu et al., 2017). Compared with studies that have examined the loss of CO_2 and CH_4 induced by SOC decomposition, there have been relatively few studies that have examined the effects of N input on soil DOC loss, and most of these have tended to focus on the effects of N input on DOC accumulation regulated by vegetation.

Given the current trend of increasing eutrophication in tidal salt marshes (Breitburg et al., 2018; Kirwan and Megonigal, 2013; Wang et al., 2018; Yuan et al., 2015), we hypothesized that the response of SOC loss to tidal action would be influenced to a certain extent by N input. As one of the most active regions of the land-ocean transition zone in the world, the Yellow River Delta (YRD) is notebly vulnerable and sensitive to the detrimental effects associated with eutrophication of coastal waters (Maryna et al., 2014; Wang et al., 2018). Thus, we considered the YRD to be an ideal areain which to investigate the interactive effects of inundation frequency and N input level on soil C loss. The main objectives of the present study were to address the following three questions: (1) how do N input and inundation frequency affect SOC loss in a tidal salt marsh? (2) Does N input influence the control of inundation frequency on SOC loss? (3) how does N input interact with inundation frequency to influence SOC loss?

2. Materials and methods

2.1. Site description

The YRD (37°35′–38°12′ N, 118°33′–119°20′ E) is located in the vicinity of Dongying City, Shandong Province, China. It has a warm temperate and continental monsoon climate with distinctive seasons and distributions of rain and heat. The annual average temperature is approximate 12.9 °C and the annual average precipitation is approximately 640 mm, a majority of which falls during the summer months (from June to September) (Han et al., 2013). At our research sites, the vegetation is relatively homogeneous and strongly dominated by *Suaeda salsa (S. Salsa)*. The predominant soil type has been characteriaed as a saline soil, and the soil texture is mainly a sandy clay loam (Yang et al., 2017). As a consequencce of the low elevation (generally below 10 m) and close vicinity to the sea, the hydrological characteristics in the research site are affected to a large extent by tidal process. The tidal pattern in this region tends to be complex, owing to difference in the terrains of the high low tidal flat, withthe high tidal flats only being flooded approximately once a month even less, whereas the low tidal flat can be flooded twice a day. The salinity of the seawater in this region ranges from 26‰ to 30‰ (Song et al., 2013). Notably, the Yellow River estuary has become severely eutrophicated, particularly with respect to the concentrations of nitrate (NO_3^-), and the levels continue to rise. The nitrate concentration in the Yellow River has been reported to exceed 200 μmol L^{-1}, and the transport flux from the Yellow River to the Bohai Sea reaches levels of up to 2×10^{10} mol $month^{-1}$ (Liu, 2012).

2.2. Experimental design

For the purposes of the present study, we examined the effects of eight treatments defined by a 2 × 4 factorial design based two tidal inundation frequencies (semidiurnal tide and lunar tide) and four N input levels (0, 100, 200, 300 μmol L^{-1}). With respect to the semidiurnal tide (ST) treatments, there were two daily tidal cycleseach of which comprised an inundation and a drainage period of 6 h, respectively. In contrast, for the lunar tide (LT) treatments, there was only a single tidal cycle each month, characterised by a 6 h inundation event followed by a 1-month drainage period. The two inundation frequency treatments were set up with a view to simulating the inundation frequency in the high and low tidal flat of the YRD. The four N input levels included a control (no N addition to simulate unpolluted water) and three levels of N application [low-N (LN), medium-N (MN), high-N (HN)] to simulate the corresponding levels of eutrophication, and each treatment was replicated 12 times. The N was provided in the form of $NaNO_3$. Analytical grade $NaNO_3$ was added to artificial seawater (a solution of 30‰ NaCl) and NO_3^--N in the eutrophic water was delivered to soil through simulated tidal action. The inundation–drainage experiments were performed in eight groups of artificial tidal tanks, each of which was constructed from two plastic tanks (120 cm × 80 cm × 50 cm), one designed to hold the artificial seawater and other for holding salt marsh soil cores. At the beginning of the inundation period, there was an approximate 5 min period during which the incubation tanks were filled with the respective artificial seawater through pipes until the water level was 10 cm above the soil surface. At the end of the inundation period, there was a corresponding 5-min period during which the water was drain out of the incubation tank back into the seawater container.

2.3. Soil cores sampling

At the study site, a total of 96 undisturbed soil cores (20 cm diameter and 30 cm long), were randomly collected in summer (August 2018) when the water level was below the ground surface, thereby enabling us to retrieve intact soil cores. The basic properties of the soil samples are listed in Table 1. The collected soil cores were placed in polyvinyl chloride (PVC) tubes (20 cm diameter and 45 cm long) and transported to the laboratory at the Yellow River Delta Ecology Research Station of Coastal Wetland, Chinese Academy of Sciences. In the laboratory, the baseof each soil core was sealed to prevent soil erosion, using a 200-mesh grid nylon filter. The soil cores were subsequently randomly allocated to each of the eight treatment groups, and placed into the corresponding incubation tanks. Among the 12 cores placed in each incubation tank, four were selected to determine CO_2 and CH_4 emission rates, whereas the remaining eight cores were withdrawn for analyses at intervals throughout the course of the incubation period. All mesocosms were preincubated for 5 days, during which the soil cores were exposed to two daily inundation cycles with unamended artificial seawater (solution of 30‰ NaCl) at 25 °C. At the end of the preincubation period, the tanks were randomly allocated to each of the eight treatments and subsequently incubated under the respective treatment conditions for 90 days at 25 °C.

2.4. Measurement of soil CO_2 and CH_4 emissions rates and soil DOC concentration

For the ST treatments, the rates of CO_2 and CH_4 emission were measured at 0, 3and 6 h after commencing inundation and drainage (a complete tide cycle) at an interval of approximately 5–10 days for ST treatment, whereas for the LT treatmens were aken at 0, 3 and 6 h after commencing inundation and at 0 h, 3 h, 6 h, 12 h, 24 h, 4 d, 7 d, 10 d, 20 d, 25 d, 30 d after commencing drainage. The liberated gases were measured using a LGR Ultraportable Greenhouse Gas Analyzer (Los Gatos Research, Inc., San Jose, USA). During period when the soil cores were inundated, the upper portions of the PVC tubes remained protruding above the surface of the water, which thus enabled us to place the analyzer chamber on the PVC tubes for gas measurement. The average value of the three replicates subjected the same treatment was used for data analysis.

Soil DOC content was determined after the 90-day incubation. Collected soil samples were initially air-dried, subsequently extracted with deionized water (soil: water = 1: 5), and then vacuum filtered through a 0.45-μm membrane after agitated and centrifuged. Finally, The DOC concentrations in the extracts were determined using a TOC-VCSN analyzer (Shimadzu Scientific Instrument, Columbia, MD).

2.5. Measurement of soil volumetric water content, salinity and microbial biomass carbon

When measuring gas fluxes, we synchronously recorded soil volumetric water content (SVWC) in the top 5 cm, 10 cm, 20 cm, and 30 cm using a soil moisture probes. For soil salinity and microbial biomass carbon (MBC) determination, we took measurements from three replicates of each incubation treatment at 10, 30, 50, 70, and 90 days. Soil salinity was measured using an electricity conduction meter (soil: water = 1: 5), whereassoil MBC was measured using the chloroform fumigation-extraction method. For the MBC analysis, two sub-samples were prepared for each of the replicate (10-g wet weight) samples, one of which was fumigated with chloroform for 48 h and the other served as the unfumigated control. Following the chloroform treatment, both fumigated and unfumigated samples were extracted with 30 ml of 0.5 mol L^{-1} K_2SO_4, agitated for 1 h on a longitudinal shaker, and then centrifuged 10 min at 4000 ^{r}pm. Samples were filtered and stored at 4 °C until analysed for SOC. The difference between the total organic carbon (TOC) of fumigated and unfumigated samples was taken to represent the MBC (Vance et al., 1987).

Table 1
Basic properties of the soil of the research site. Data represent the means ± standard errors (n = 3). Different lowercase letters indicate significant differences (one-way ANOVA, $P < 0.05$) among different soil layers.

Soil properties	Soil depth (cm)			
	0–5	5–10	10–20	20–30
pH	9.03 ± 0.02 ab	8.96 ± 0.03bc	9.07 ± 0.03a	8.98 ± 0.04ac
Salinity (mg g^{-1})	19.32 ± 0.14a	19.19 ± 0.61a	18.52 ± 1.1a	18.32 ± 0.73a
TC (mg g^{-1})	16.53 ± 0.51a	16.92 ± 0.55a	17.51 ± 0.35a	16.51 ± 0.21a
SOC (mg g^{-1})	3.27 ± 0.04a	3.21 ± 0.12 ab	3.03 ± 0.03bc	2.88 ± 0.04c
DOC (mg kg^{-1})	102.99 ± 3.3a	80.66 ± 2.17b	73.59 ± 4.48bc	66.44 ± 2.28c
TN (mg g^{-1})	0.35 ± 0.04a	0.4 ± 0.03a	0.41 ± 0.06a	0.35 ± 0.02a

TC: Total carbon, SOC: Soil organic carbon, DOC: Dissolved organic carbon, TN: Total nitrogen.

2.6. Statistical analysis

Data are presented as the means of three replicates with standard errors (SE). Statistically significant differences in cumulative CO_2 and CH_4 emissions and reductions in DOC among treatments were analysed using one-way ANOVA followed by least significant difference (LSD) tests ($P < 0.05$). Mixed effects models with multi-factor ANOVA were used to examine the effects of the interactions among N input, frequency of tidal inundation and soil depth on SOC losses. The relationships among CO_2 and CH_4 emission and DOC concentrations with N input levels and soil properties were examined using linear regressions. All the data analyses were performed using Statistical Product and Service Solutions 20.0 Software (SPSS Inc., Chicago, USA). Figures were produced using Sigmaplot 12.5.

3. Results

3.1. The effects of inundation and N input on CO_2 and CH_4 emissions

In all incubation treatments, CO_2 and CH_4 emissions generally tracked the process of inundation and drainage. During the inundation period, the rates of both soil CO_2 and CH_4 emission remained stable and relatively low (approximately 2 mg CO_2–C g^{-1} soil h^{-1} and 2 μg CH_4–C g^{-1} soil h^{-1}, respectively). Following drainage, however, among all treatments, rapid pulses in CO_2 and CH_4 were produced over a short period time (Fig. 1). Moreover, in LT treatment, whereas the rate of CO_2 emission increased with a decrease in SVWC, that of CH_4 peaked approximately 12 h after drainage and thereafter declined with a reduction in SVWC (Fig. 1a and c). Further, we found that the mean SVWC and total CO_2 and CH_4 emissions during the drainage period were significantly higher than those recorded during the inundation period ($p < 0.05$, Fig. 1b, d and f), although the observed differences were smaller in the ST treatments than those in the LT treatment (Fig. 1).

For all N input treatments, cumulative CO_2 emissions (4.97–5.68 g CO_2– C m^{-2} in ST treatment and 12.94–15.49 g CO_2– C m^{-2} in LT treatment) were significantly lower than those in the control (6.72 in ST treatment and 18.62 g CO_2– C m^{-2} in LT treatment, Fig. 2a). Furthermore, we found that cumulative CO_2 emissions decreased linearly with increasing N input level (Fig. 2b, $p < 0.01$) with the regression slope value of the LT treatment considerably larger than that of the ST treatment (Fig. 2b). Similarly, cumulative CH_4 emissions decreased linearly with increasing N input level in the ST treatments (Fig. 2d, $p < 0.01$) whereas in the LT treatments, N input promoted a significant increase in CH_4 emissions, although the cumulative CH_4 emissions decreased linearly with an increasing level of N input (Fig. 2c and d, $p < 0.01$). Considering the two inundation treatments together, we found that N input alone had no significant effects on CH_4 emissions (Table 2, $p > 0.05$), whereas we observed a significant interactive effect of inundation frequency and N input on CH_4 emissions (Table 2, $p < 0.001$).

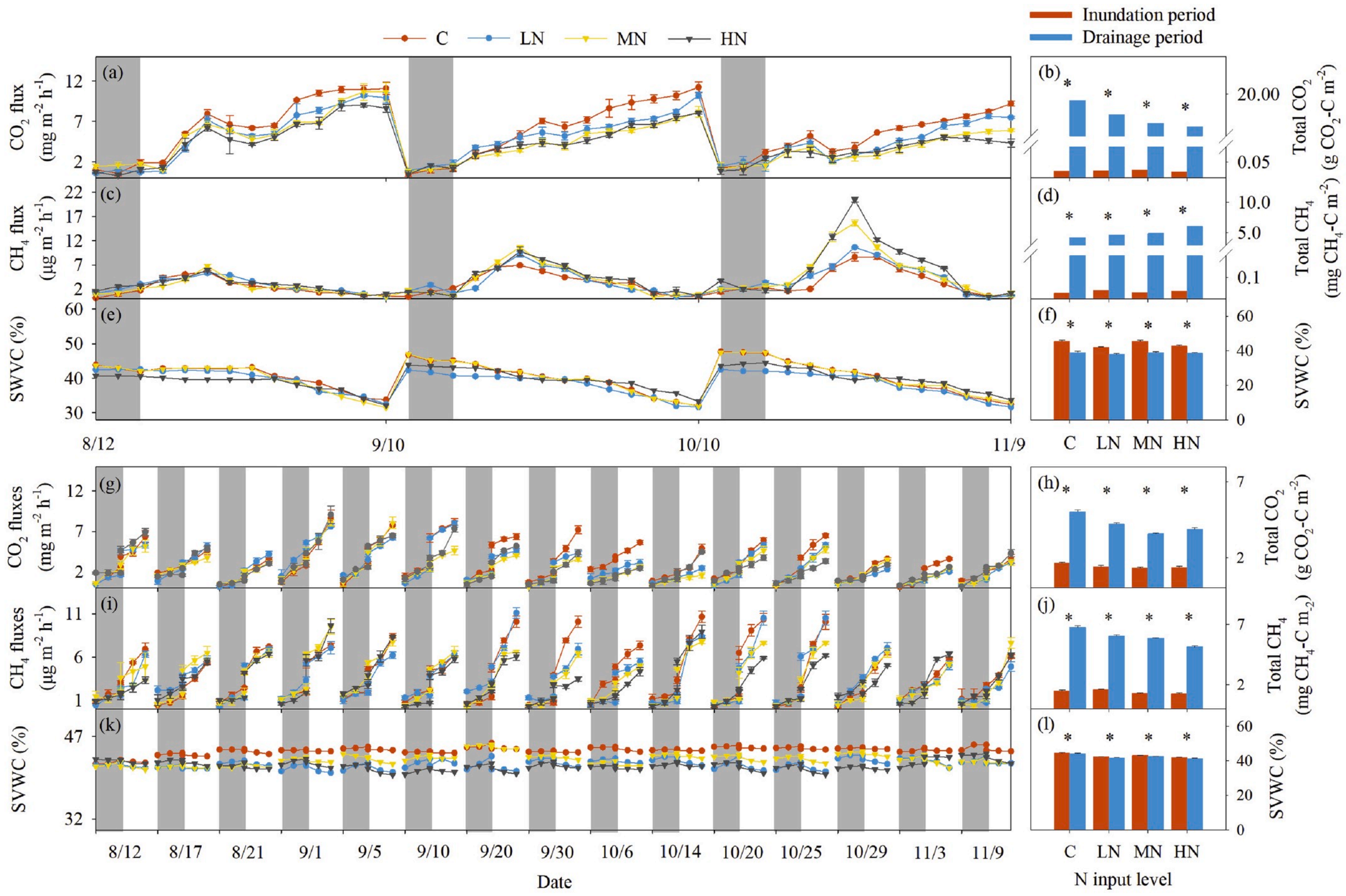

Fig. 1. Temporal changes in CO_2 emission rates (a and g), CH_4 emission rates (c and i) and soil volumetric water content (e and k) at 5 cm depth during 90 days incubation affected by N input under two inundation frequency treatments. N input levels included C (control) of 0 μM, LN (low-N input) of 100 μmol L^{-1}, MN (medium-N input) of 200 μmol L^{-1}, HN (high-N input) of 300 μmol L^{-1}. Gray shaded areas correspond to inundation periods for 6 h each time. The histogram represents cumulative CO_2 emissions (b and h), cumulative CH_4 emissions (d and j), average soil volumetric water content (f and l) at 5 cm depth in drainage period and inundation period, respectively. Values represent the means of three replicates, while the vertical lines indicate standard errors. Star on the top of bars represent × significant differences ($P < 0.05$).

3.2. *The effects of inundation and N input on DOC loss*

DOC loss was found to be strongly affected by the frequency of inundation (Fig. 3). Among the four layers of soil we examined, reductions in DOC in the ST treatments were found to be higher than those in the LT treatments. Moreover, whereas N input had no appreciable effect on DOC loss in LT treatments, we found that N inputs significantly inhibited DOC loss in the ST treatments. Overall, surface soil was found to be the main source of DOC loss, particularly in the LT treatments. Collectively, our observations indicated that inundation frequency and soil depth were the key factors controlling the reduction in DOC, whereas the defferent levels of N input and the interactions among N input, inundation frequency, and soil depth appeared to have no significance effects (Table 2).

3.3. *Relationships between vertical SOC loss and DOC reduction*

In the LT treatments, we found that vertical SOC loss, which represents the combined emissions of CO_2 and CH_4, was linearly correlated with a reduction in soil DOC (Fig. 4, $r^2 = 0.39$, $p < 0.05$), whereas no significant relationships were detected in ST treatment (Fig. 4, $p > 0.05$).

3.4. *Relationships between CO_2 and CH_4 emission rates and soil moisture and salinity*

In the LT treatments, SVWC was found to decline with time following drainage, whereas both soil salinity and MBC showed gradual increases, with variations being most pronounced in the surface soil (Fig. 5a, b and c). In contrast, in the ST treatment, we detected no obvious change in SVWC, soil salinity or MBC during the entire incubation period (Fig. 5d, e and f).

We also observed a negative relationship between SVWC and CO_2 emission rates in all N input treatments (Fig. 6a). A comparison of the parameters revealed that the LN and MN treatments had the effect of enhancing the rate of CO_2 emission in response to soil moisture, whereas the HN treatments had the opposite effect. In contrast, in all N input treatments, we observed that the rates of CH_4 emission were significantly increased with increasing, and that threr was also a significant increase in moisture sensitivity in response to an increase in the input level of N (Fig. 6b). However, we detected no obvious relationship between the rates of CO_2 and CH_4 emissionand soil salinity (Fig. 6c and d).

4. Discussion

4.1. *Effects of inundation and N input on CO_2 and CH_4 fluxes in a tidal salt marsh*

In this study, we found that inundation significantly suppressed the

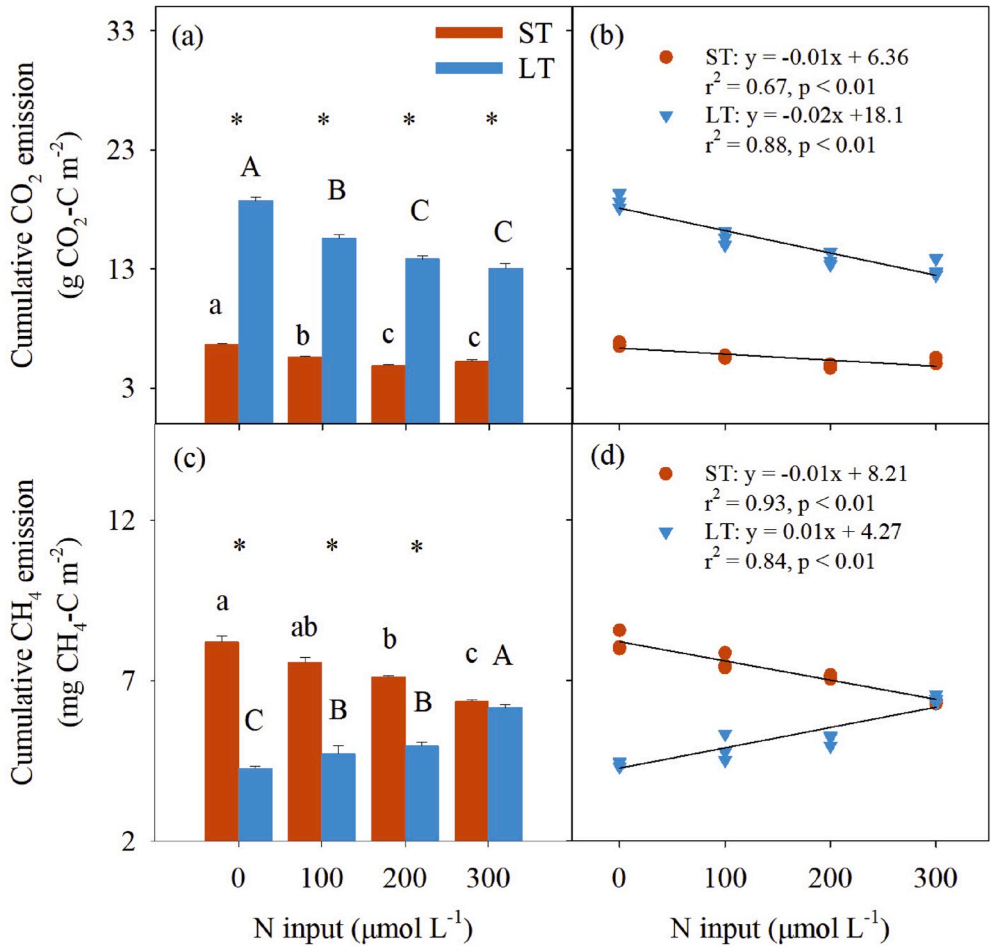

Fig. 2. Cumulative CO_2 (a) and CH_4 (c) emissions for the 0 μmol L^{-1}, 100 μmol L^{-1}, 200 μmol L^{-1}, 300 μmol L^{-1} treatments in the two inundation frequency treatments, and correlations of (b) cumulative CO_2emission and (d) cumulative CH_4 emission with N input gradient in two inundation treatments. ST and LT represent semidiurnal tide and lunar tide treatment, respectively. Vertical bars represent the standard errors of three replicates. Star on the top of bars represent significant differences between the two inundation treatments and different letters indicate differences between the four N input level treatments ($P < 0.05$). The scattered points represent data measured in each mesocosm.

Table 2
ANOVA results for the mixed effects model testing the N input (N) × inundation frequency (I) × soil depth (D) interaction effects on cumulative soil CO_2–C (g C m^{-2}) and CH_4–C (mg C m^{-2}) emissions and reduction of soil DOC (mg kg^{-1}).

	CO_2–C loss	Effect size[a]	CH_4–C loss	Effect size	DOC loss	Effect size
	F-value		F-value		F-value	
N	76.95***	0.94	1.07	0.17	28.42***	0.58
I	2708.57***	0.99	579.49***	0.97	230.26***	0.88
D					310.46***	0.94
N*I	23.22***	0.81	68.71***	0.93	20.66***	0.50
N*D					3.64**	0.34
I*D					8.43***	0.29
N*I*D					6.294***	0.47

** represents significant at $P < 0.01$; *** represents significant at $P < 0.001$.
[a] Effect size is determined by partial Eta^2 provided by SPSS.

emissions of CO_2 and CH_4 in our tidal salt marsh mesocosms, which is consistent with the findings of Moyano et al. (2013), who observed that virtually no gas diffusion occurs in water-saturated soils, owing to a disconnection of conduits. (Moyano et al., 2013). Inundation would limit not only the emission of CO_2 and CH_4, but also the inward diffusion of oxygen (Cook and Knight, 2003; Jimenez et al., 2012), and in this regard, a previous study on wetlands found that soil respiration increases during periods of frought, as a consequence of an increase in the depth of soil oxygenation (Malone et al., 2013). However, the production of CH_4 is a predominantly anaerobic process (Liu et al., 2017; Olsson et al., 2015), and although inundation inhibited CH_4 diffusion from the soil, the anoxic conditions are favourable in terms of methanogenesis. We found that variation in soil moisture in the vertical direction was the main factor controlling CO_2 and CH_4 emissions. In the LT treatment, with progression of the drying phase, there was a gradual reduction in soil moisturein the surface soil and a concomitant accumulation of salt (Han et al., 2018). Generally, the evaporation of moisture is conductive to the decomposition of SOC through its effects in enhancing the penetration of oxygen into soil pores (Olsson et al., 2015), whereas an increase in salinity has been found to have a negative effect on microbial activity and SOC decomposition (Qu et al., 2018). In the present study, we observed a gradual increase in the MBC content of surface soil with time during the drying phase, thereby indicating a positive effect of a reduction in moisture on CO_2 emission that may exceed the negative effect of drying that promotes an increase in salinity.

Our results also revealed that cumulative CO_2 emissions decreased linearly with increasing N input level (Fig. 2), which is consistent withthe findings of some previous studies that have indicated that N addition inhibits soil heterotrophic respiration (Wang et al., 2017; Yan et al., 2018), although not with others (Chen et al., 2018; Zhang et al., 2014). C availability or C use efficiency is a crucial driver regulating soil CO_2 emissions in response to N input (Liu et al., 2018). Soil microbial C use efficiency tends to be relatively low in N-limited ecosystems, and under such circumstances, it is necessary for soil microbes to allocate larger amounts of energy and more C to obtain and assimilate N (Spohn et al., 2016). Given that the availability of N in promoted after N input, there would be a subsequent increase in the C use efficiency of soil microbes, and thus less C would be decompose (Fisk et al., 2015; Spohn et al., 2016). A series of studies have found that N input leads to a reduction in soil C availability via the suppression of SOC decomposition

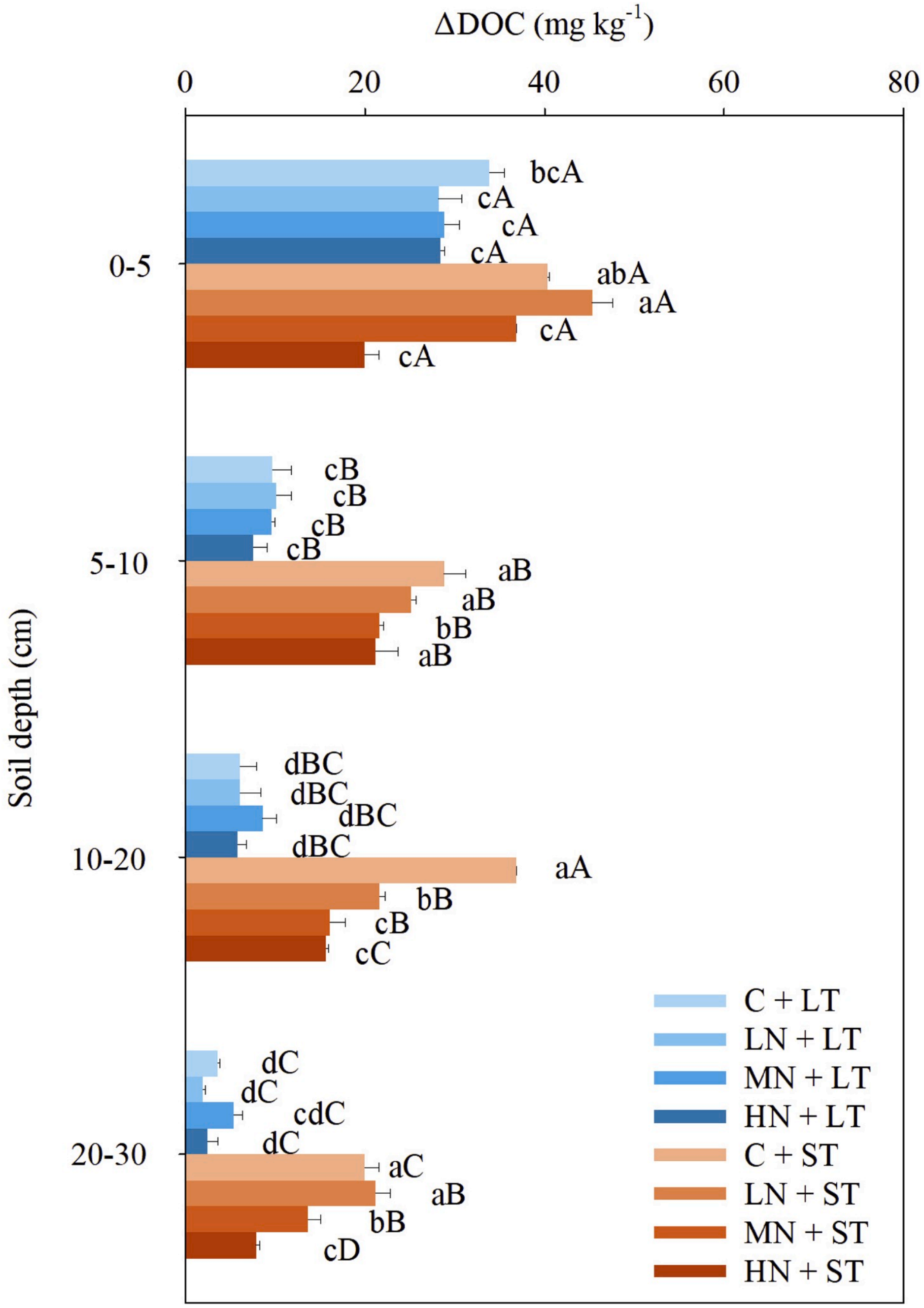

Fig. 3. Comparison of soil DOC reduction (ΔDOC) among different soil depths and different treatments. Horizontal bars represent the standard errors of three replicates. Different uppercase letters indicate significant differences among soil depths for the same treatment ($p < 0.05$); different lowercase letters indicate significant differences among different treatments at the same soil depth ($p < 0.05$).

(Jian et al., 2016; Riggs et al., 2015). Similarly, inconsistent results have tended to be obtained with respect to the effects of N input on CH_4 emissions in different ecosystems. In the present study, we fpund that the effect of N input on CH_4 emission underwent a transition from positive to negative with an increase in the frequency of inundation (Bodelier and Laanbroek, 2004), thereby indicating that the relationship between changes of N input level and CH_4 emissions are non-linear.

Although we found that N input had no significant effects on CH_4–C loss, we detected a significant interactive effect of N input and inundation on the loss of CH_4–C, which would be attributable to the contrasting responses of CH_4 emissions to N input in the two different tide frequency treatments (Fig. 2d). We found that the positive effects on CH_4 emissions among different N inputs in the LT treatment were offset by negative effects that occurred in the ST treatment. Accordingly, when assessing CH4 emissions in tidal salt marshes, it is necessary to take into consideration the response of soil CH_4–C loss to N input under different tidal frequencies for an accurate modeling of the ecosystem C cycle (Hu et al., 2019). In addition, we found that N input altered the rates of soil CO_2 and CH_4 emissions in response to changes in soil moisture (Fig. 6). Notably, N input appeared to have the effect of enhancing the sensitivity of CO_2 and CH_4 emissions to soil moisture, which is consistent with the findings of previous studies that have indicated that N addition enhances the response of soil respiration to fluctuations in soil moisture (Chen et al., 2019; Zhang et al., 2017). These results thus indicate that the effect of N input on the soil moisture control of CO_2 and CH_4 emissions are dependent to a large extent on the input level of N.

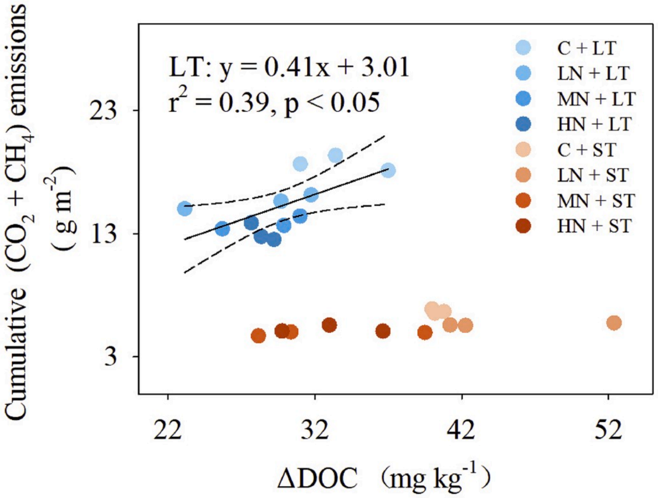

Fig. 4. Correlations of cumulative ($CO_2 + CH_4$) emissions with DOC reduction (ΔDOC) in the surface layer (0–5 cm). Dash lines indicate the bounds of the 95% confidence intervals for the regression equations.

4.2. *Effects of inundation frequency and N input on DOC loss in a tidal salt marsh*

Our results revealed that inundation frequency is a key factor influencing the loss of DOC in tidal salt marsh. In tidal wetlands, the flux in DOC is largely dependent on hydrological dynamic (Lee et al., 2018), and strong positive correlations have been detected between the fluxes of DOC and water in previous field studies (Borken et al., 2011; Wu et al., 2013), whereas in laboratory studies lager amounts of DOC have been found to be released from the soil subjected to a greater frequency of leaching (Lee et al., 2018). Hence, together with our findings, these observations provide compelling evidence that an increased in tidal frequency, as a consequence of rising sea levels, may result in considerably lager amounts of DOC being exported from land to sea. In contrast, we found that N input had a negative effect on ST treatments and no appreciable effect on LT treatments. As one of the most active and mobile C forms, DOC is generally considered to be the source of C substrate for microbial activities (Liu et al., 2017; Luca et al., 2006), and thus the observed decrease in DOC loss in response to N input is probably associated with a reduction in the decomposition of SOC. Compared with the frequency of inundation, we found that N input had a less pronounced effect on soil DOC loss, and, moreover, we detected a strongly antagonistic effect of increasing N input and increasing inundation frequency on DOC loss (Table 2, Fig. 3). Notably, we observed that N input tended to reduce the difference in DOC loss between the two assessed inundation frequencies. Accordingly, we anticipate that N input would weaken the effects of increasing inundation frequency on the lateral loss of DOC induced by future rises in sea level.

In the present study, we found that the reduction in DOC loss in response to inundation was also dependent on soil depth. In this regard, surface soil is invariably strongly flushed strongly flushed by tidal activity, causing a loss of DOC in the top 5 cm of soil (Zhao et al., 2018). Moreover, due to the permanent anaerobic condition in subsurface soils, decomposition of DOC would be suppressed due to a reduction in the activity of aerobic microorganisms in this oxygen-limited environment (Malone et al., 2013). This would also explain why SOC can be sequestered and buried in the soil profile.

4.3. *Relationship between soil DOC reduction and vertical SOC loss*

Our results indicated that total CO_2 and CH_4 emissions in the LT treatments were significantly positively related to a reduction in soil DOC, whereas we detected no obvious correlations in the ST treatments. Given that the presence of soil water is essential for substrate assimilation by soil microorganisms, and that soil organic matter can only be decomposed after dissolution, soil DOC serves as the main C substrate for microorganisms (Marschner and Kalbitz, 2003). In the LT treatments,

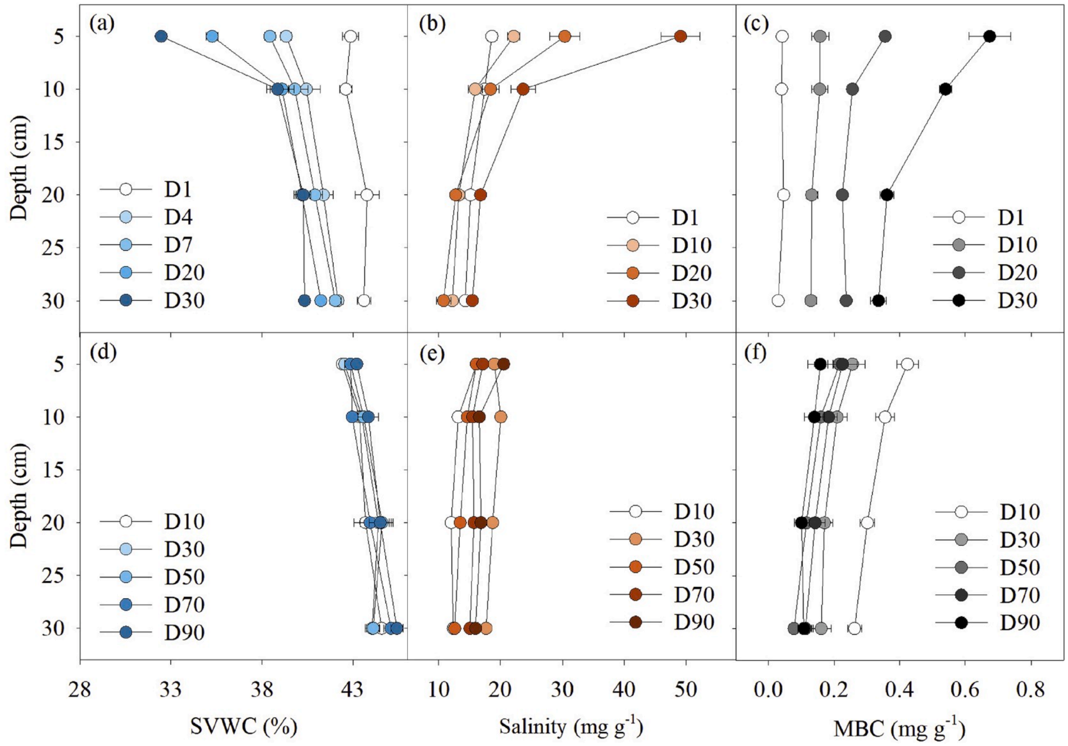

Fig. 5. The variations of SVWC (a), soil salinity (b) and SMBC (c) with soil depth in several days after drainage in LT treatment. The variations of SVWC (d), soil salinity (e) and SMBC (f) with soil depth in several days during the whole incubation period. Horizontal bars represent the standard errors of replicates.

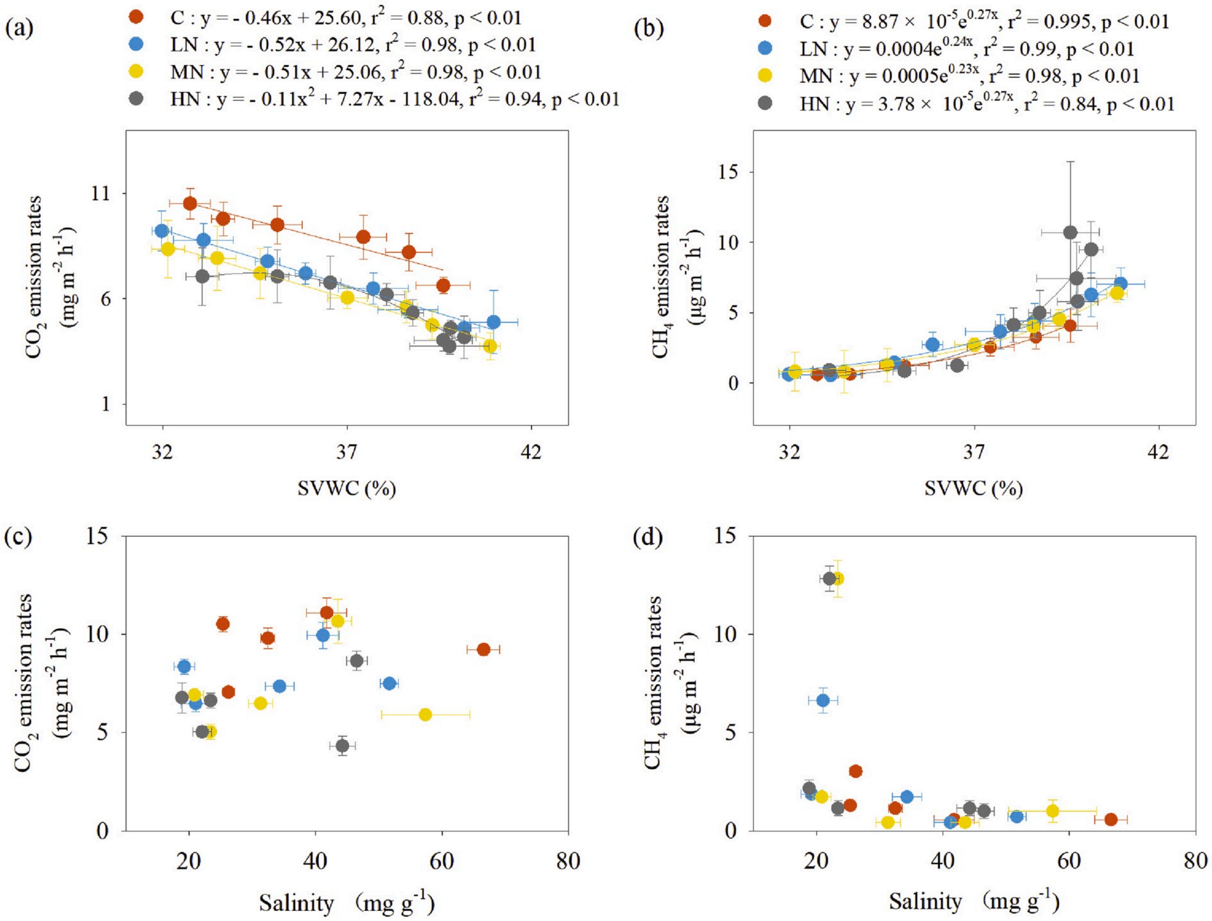

Fig. 6. Correlations of (a) CO_2 emission rates with SVWC and (b) CH_4 emission rates with SVWC under different N input levels during drainage period in lunar tide treatment. C, LN, MN, HN represent low N, medium N, high N treatment, respectively. The points were calculated as the means of nine replicates (3 replicate mesocosms × 3 tidal cycles) at every N input level. The error bar represents standard error of replicate.

the long-term aerobic condition proved beneficial with respect to DOC degradation, and a large proportion of the soil DOC was converted to CO_2 and subsequently released into the atmosphere. Moreover, as a consequence of the low tidal frequency in these treatments, lateral loss of SOC was markedly reduced, whereas vertical SOC loss increased with increasing reduction of DOC. A strong correlation between CO_2 production and DOC has also been observed in numerous previous studies (Fellman et al., 2017; Liu et al., 2017). Although the production of CH_4 tends to be more complex than that of CO2 and is controlled by a multiplicity of other factors, the total emissions are generally insufficiently high to influence the relationship between total vertical SOC loss and soil DOC (Liu et al., 2017). In contrast, in ST treatments, only a small fraction of soil DOC was utilised by microorganisms owing to the large water flux and the associated anaerobic conditions. Consequently, the relationship between soil DOC and vertical SOC loss would differ to varying extents depending on the level of soil moisture.

4.4. Features, limitations and prospectives of the present study

On the basis of mesocosm experiments in which we examined the effects of defferent concentrations of nitrate in water and frequencies of inundation, we sought to elucidate the mechanisms whereby water body eutrophication and rising sea levels would influence SOC losses in a tidal salt marsh in the YRD. Using this approach, we were able to limit the variability of other control factors, such as the depth and duration of inundation, air temperature, and SOC content. Furthermore, it provided a convenient system for measuring soil CO_2 and CH_4 emissions at any time in the laboratory, which enabled us obtain data before and after inundation. Moreover, using soil moisture probes, we were able to perform measurements of SVWC simultaneously with our analysis of gas emission. Although previous studies have assessed the effect of tidal inundation on soil CO_2 and CH_4 emissions using eddy covariance technique (Han et al., 2018; Hong et al., 2018; Kathilankal et al., 2008), the approach adopted in the present study has the benefits of convenience and high efficiency, along with the provision of high time resolution data resources. Consequently, it is deemed more applicable to long-term detection, based on measurements that can be obtained with minimal disturbance. Despite these multiple advantages, however, the present study does have certain limitations and deficiencies. Notably, we did not take into consideration the biochemical modulations attributable to plants and microorganisms. Microorganisms are key drivers of the decomposition of SOC, whereas plants are not only a significant source of SOC but also play important roles in regulating the structure and function of the soil microbial community (Hu et al., 2019). Furthermore, the composition and coverage and degree of coverage of vegetation, which are influence to a considerable extent by N input, may have a substantial effect on soil DOC accumulation and loss in tidal salt marshes. Therefore, further studies that take into consideration the respective contributions of plants and microorganisms, are necessary to gain a more comprehensive insight into how biotic and abiotic factors might affect tSOC loss in response to rising sea levels and increased water eutrophication. In addition, in the present study, we only considered the short-term effects of N input and inundation on SOC loss in tidal salt marshes and it is quite probable that the long-term effects of N input on SOC loss will differ from those observed over a short period of time (Deegan et al., 2012; Zhou et al., 2014). Thus, it will be necessary to conduct longer-term in situ experiments to investigate the underlying mechanisms on a larger time scale.

5. Conclusion

In this study, we demonstrated that inundation of a simulated salt marsh environment inhibited CO_2 production and stimulated CH_4 formation, due to high soil moisture content and anaerobic conditions. N input suppressed soil CO_2 emission in both LT and ST treatments, whereas N input promoted an increase and decrease in CH_4 emissions in the LT and ST treatments, respectively, thereby indicating that tidal frequency is a key factor influencing CH_4 in response to N input. During the inundation period, we observed reductions in the rates of soil CO_2 emissionand increases in those of CH_4 with rising moisture content; however, N input was found to alter the rates of CO_2 and CH_4 emission in response to variations in soil moisture. Frequent inundation was found to result in greater soil DOC loss, which can be ascribed to the large water flux induced by the frequent tidal activity. Furthermore, whereas in the ST treatments, N input inhibited DOC loss, it had no appreciable effects on DOC loss in the LT treatments. In addition, we found that the reductions in soil DOC in the LT treatments were positively correlated with total SOC loss as CO_2 and CH_4, thereby indicating that DOC is the main source of substrates for soil microorganisms. In conclusion, the findings of the present study reveal a strong interaction between N input and inundation frequency with respect to salt marsh C transformations, with differences in SOC loss being observed in response to different coupling treatments. Nevertheless, it should be emphasized that laboratory experiments have certain limitations and efficiencies. Notably, the mesocosms used in the present study were devoid of vegetation, and it is highly probable that the presence of plants would have a prominent influence (either strengthening or weakening) on the effects of N input on SOC loss. Accordingly, field experiments should be conducted in the future to investigate the long-term effects of N input and different tidal frequencies on ecosystem C exchange in tidal salt marshes.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Juanyong Li: Writing - original draft, Writing - review & editing, Investigation, Methodology, Data curation, Conceptualization. **Guangxuan Han:** Conceptualization, Funding acquisition, Project administration, Writing - review & editing. **Mingliang Zhao:** Methodology, Investigation, Visualization, Formal analysis. **Wendi Qu:** Formal analysis, Investigation, Visualization. **Ming Nie:** Formal analysis, Writing - review & editing, Project administration. **Weimin Song:** Formal analysis, Writing - review & editing, Project administration. **Baohua Xie:** Formal analysis, Writing - review & editing, Project administration. **Franziska Eller:** Formal analysis, Writing - review & editing.

Acknowledgements

This research was funded by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA23050202) and the National Nature Science Foundation of China (41671089). We are grateful for the support from the Yellow River Delta Ecological Research Station of Coastal Wetland, CAS, and also thank Xiaojie Wang, Xiaojing Chu, Xiaoshuai Zhang, Wenjun He and two anonymous reviewers of their expert advice and fruitful comments.

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