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Methane Uptake and Nitrous Oxide Emission in Saline Soil Showed High Sensitivity to Nitrogen Fertilization Addition

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Abstract: Saline soils can significantly affect methane (CH₄) and nitrous oxide (N₂O) in atmospheric greenhouse gases (GHGs). However, the coupling effect of nitrogen fertilization addition and saline soils on CH₄ uptake and N₂O emissions has rarely been examined under various salinity conditions of soil. In this study, the effects of nitrogen fertilization addition on CH₄ and N₂O fluxes under different salinity conditions of soil in Hetao Irrigation District, Inner Mongolia, were investigated by on-site static chamber gas chromatography. A slightly saline soil (S₁) (Electrical Conductivity: 0.74 dS m⁻¹) and a strongly saline soil (S₂) (EC: 2.60 dS m⁻¹) were treated at three levels of nitrogen fertilization: a high fertilization rate of 350 kg N ha⁻¹ (H), a low fertilization rate of 175 kg N ha⁻¹ (L), and no fertilizer (control treatment, referred to as CK). Nitrogen application was the important factor affecting N₂O emissions and CH₄ uptake in saline soil. The CK, L, and H treatments exhibited a cumulative CH₄ uptake of 156.8–171.9, 119.7–142.0, and 86.7–104.8 mg m⁻² in S₁, 139.3–176.0, 109.6–110.6, and 68.5–75.4 mg m⁻² in S₂, respectively. The cumulative N₂O emissions under the L and H treatments in S₂ were 44.1–44.7%, and 74.1–91.1% higher than those in S₁. Nitrogen fertilizer application to saline soils reduced CH₄ uptake and promoted N₂O emission in the Hetao Plain, Inner Mongolia. Our results indicate that mitigating soil salinity and adopting appropriated fertilizer amounts may help to cope with global climate change.



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Keywords: CH₄ uptake; N₂O emission; nitrogen fertilization; soil salinity

1. Introduction

Global warming caused by greenhouse gases (GHGs) from carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) has become a major environmental problem facing the world. The radiative forcing of nitrous oxide (N₂O) and methane (CH₄) is of greater concern because of their 265 and 34 times higher global warming potential, respectively, compared to carbon dioxide (CO₂), on a 100-year time scale [1]. From 1990 to 2005, global CH₄ and N₂O fluxes from agricultural activities increased by 17% [2], with agricultural activities accounting for 47% and 58% of anthropogenic emissions of atmospheric CH₄ and N₂O, respectively, in 2005 [3]. The increase in the GHGs (CH₄ and N₂O) emitted by agricultural production is closely relevant to changes of soil salinity and nitrogen fertilizer in land use.

Previous studies primarily focused on the effects of fertilization on CH₄ and N₂O fluxes in non-saline and non-alkaline soils in agricultural ecosystems [4–6]. However, the high salt content in soil causes microbial osmotic stress and specific ion toxicity in soil [7], which affects soil physicochemical properties and microbial activities related to soil carbon and nitrogen cycles, thereby affecting CH₄ and N₂O fluxes in saline soils. The CH₄ uptake and N₂O emissions depend on the amount of salt content and are produced via multiple soil-nitrogen transformation pathways, such as nitrification and denitrification processes

being affected by salt content in the soil [8]. Limited consideration has been given to the effects of nitrogen fertilizer on CH₄ uptake and N₂O emission in saline soil [9].

Saline soils are widespread and rapidly increasing in more than 120 countries, owing to climate change, seawater intrusion, and inappropriate irrigation and drainage management [10]. Saline soils currently account for approximately 20% of the world's arable land [11]. China has 99 million hectares of saline soils and ranks third globally in terms of saline soil area. The area of high saline soil in the Hetao Irrigation District in Inner Mongolia reaches approximately 797,300 ha because of excessive use of chemical fertilizer and flood irrigation to wash and drain the salts [12]. It is essential to determine the effect of salinity on soil carbon and nitrogen cycling, improve nitrogen utilization in saline soils, reduce the environmental impact of nitrogen losses, ensure rational use of saline soils, and reveal the interactive effects of fertilization and soil salinity on soil carbon and nitrogen cycling [13,14]. The observations of the CH₄ and N₂O fluxes in nitrogen-fertilized saline soils are few in situ studies. This limits the scientific estimation of total CH₄ and N₂O fluxes in arable saline soils, hindering the establishment of technical approaches for reducing GHGs emissions in saline soils.

The main objectives of this study are to (i) evaluate how fertilization and soil salinity is already affecting CH₄ uptake and N₂O emission from saline soil, (ii) assess the relationship between physico-chemical properties of soil and CH₄ uptake or N₂O emission to clarify the potential regulatory mechanisms in fertilized saline soil, and (iii) identify uncertainties and knowledge gaps and suggest future directions in the cumulative estimation of CH₄ and N₂O in nitrogen-fertilized arable saline soils for improving the research of soil salinity and nitrogen fertilization.

2. Materials and Methods

2.1. Study Area

The study area is located at Urat Front Banner, the most representative agricultural planting area of saline soils in China (40°28' N, 108°11' E). Urat Front Banner is in an arid region of northwest China, with a historical mean annual temperature of 3.6–7.3 °C variation, precipitation of 200–260 mm, an annual mean sunshine duration of 3202 h, a frost-free period of approximately 120 days per year, and an annual mean evaporation of 1900–2300 mm [15].

The monthly mean atmospheric temperature in April–November was consistent in 2015 and 2016, exhibiting a gradual increase from April to July–August, followed by a slow decrease from September to November. There was an inter-annual difference in atmospheric precipitation, with a lower intensity and frequency of atmospheric precipitation in 2016 (Figure 1). These data were obtained from a meteorological station. The station is 30 m away from the research field.

Two types of arable saline soils with different salinity conditions were selected as the test soils; S1 is a slightly saline soil with an EC of 0.74 dS m⁻¹ and S2 is a strongly saline soil with an EC of 2.60 dS m⁻¹. The soil salt content in the treatments is presented in Table 1, the criteria for the classification of the saline soils are listed in Table 2, and the physicochemical properties for S1 and S2 soils in addition to the soil salt content are listed in Table 3. The distance is 100 m between plots S1 and S2. The two plots have the same soil type, slope, and area (~5 ha). The type of nitrogen fertilizer used was diammonium phosphate and urea in each plot, including three treatment s: (1) no fertilization (S1CK and S2CK), (2) low-rate fertilization (175 kg N ha⁻¹) (S1L and S2L), and (3) high-rate fertilization (350 kg N ha⁻¹) (S1H and S2H). Each treatment in plots S1 and S2 was conducted in 100 m² and placed randomly by triplicate subplots.

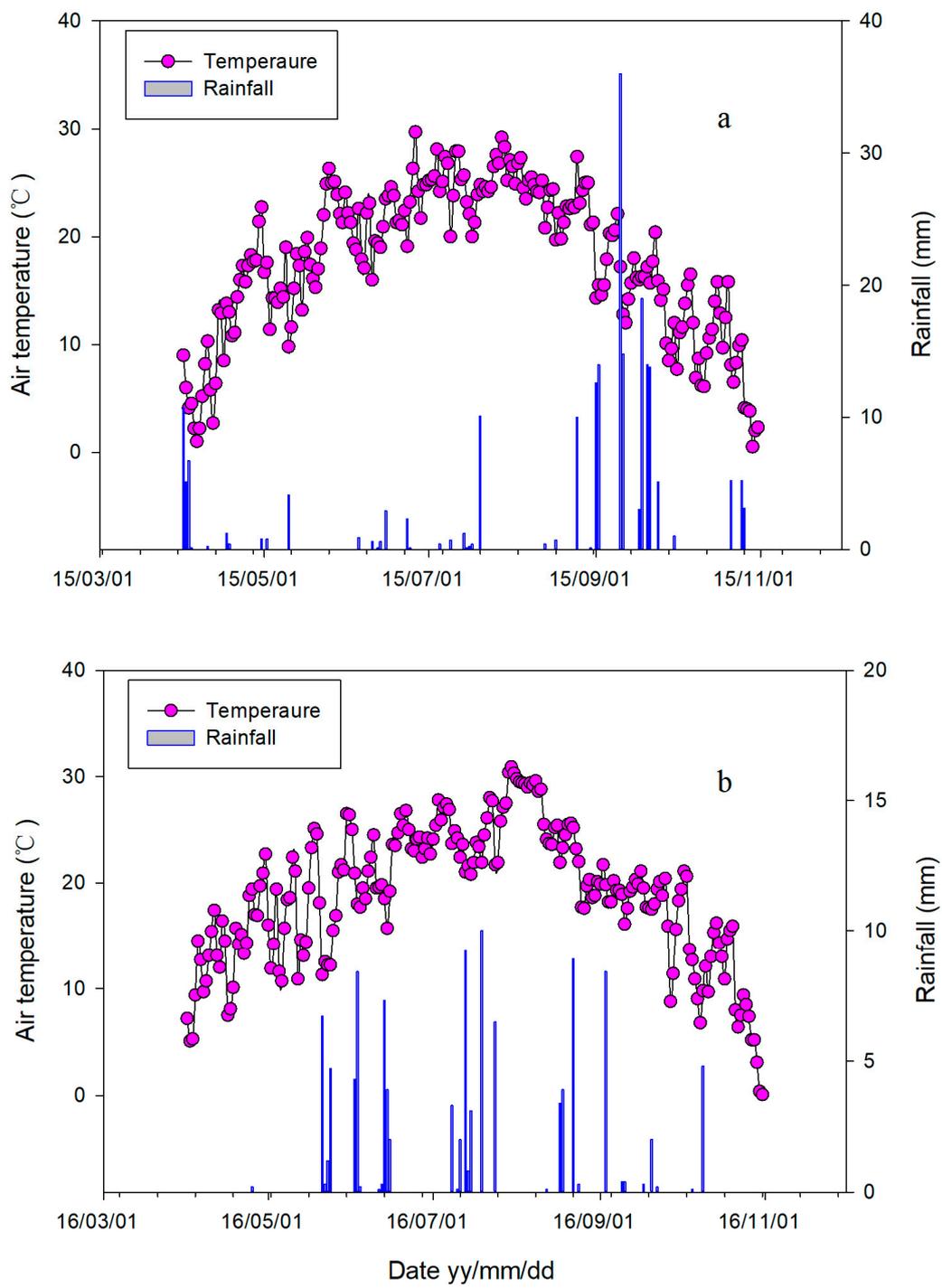


Figure 1. Changes in daily average temperature and precipitation (a) for 2015 and (b) for 2016.

Table 1. Soil salt content in the different soils in the study site (%).

Soils	K ⁺ /(%)	Na ⁺ /(%)	Ca ²⁺ /(%)	Mg ²⁺ /(%)	SO ₄ ²⁻ /(%)	CO ₃ ²⁻ /(%)	HCO ₃ ⁻ /(%)	Cl ⁻ /(%)	Total Salt Content/(%)
S1	0.002	0.009	0.014	0.0056	0.013	0.000	0.064	0.010	0.120
S2	0.006	0.120	0.083	0.045	0.390	0.000	0.048	0.140	0.830

Table 2. Soil salinization classification index [16].

Time Treatments	Soil Salt Content (%)					Saline Soil	Saline Type
	Non Salinization	Low	Medium	High			
Coastal and semi-humid, semi-arid and arid Regions	<0.1	0.1–0.2	0.2–0.4	0.4–0.6 (1.0)	>0.6 (1.0)	HCO ₃ ⁻ +CO ₃ ²⁻ , Cl ⁻ , Cl ⁻ -SO ₄ ²⁻ , SO ₄ ²⁻ -Cl ⁻	
Semi desert and desert area	<0.2	0.2–0.3 (0.4)	0.3–0.5 (0.6)	0.5(0.6)–1.0 (2.0)	1.10 (2.0)	SO ₄ ²⁻ , Cl ⁻ -SO ₄ ²⁻ , SO ₄ ²⁻ -Cl ⁻	

Table 3. The physicochemical properties for S1 and S2 soils in addition to the soil salt content.

Soils	Texture			pH	OC (g kg ⁻¹)	TN/ (mg kg ⁻¹)	TP/ (mg kg ⁻¹)	BS/ (%)	CEC (cmol kg ⁻¹)
	Clay (%)	Silt (%)	Sand (%)						
S1	33.7	38.2	28.1	8.11	9.6	32.8	0.55	61.7	10.5
S2	34.1	40.5	25.4	8.79	8.3	22.1	0.85	70.3	10.1

Note: OC: Organic C; TN: Total N; TP: Total P; BS: Base saturation; CEC: cation exchange capacity.

Each plot was planted with sunflower (*Helianthus annuus*) in May every year. The land was plowed with a deep tiller before planting. The sunflower field was irrigated by the Yellow River in China. The irrigation type used was flood irrigation. The salts leach into the soil at an intensity of 400 mm during the first irrigation and are transported into the ground for plant growth. When there is no water ponding, the farmers can enter the farmland to cultivate and the sunflowers are planted. Diammonium phosphate was applied as the base fertilizer. The amount applied as base fertilization in each treatment accounts for 45% of the total nitrogen fertilizer in the whole growth period. Urea was applied as top-dressing fertilizer. The 55% of the total nitrogen fertilizer in the whole growth period is applied at the top-dressing stage. The fertilizers were applied to the soil by broadcasting. In addition to N, P, and K, no soil correction was applied. Except for the irrigation before planting, no irrigation is carried out at other times during the growing season of crops. The sunflowers were harvested in September every year.

2.2. Gas Sample Collection and Determination

Three fixed sampling points were set within each replicate sub-plot of each study plot. Atmospheric air samples were collected using the static chamber. The chamber size was 0.5 m (length) × 0.5 m (width) × 0.5 m (height). These static chambers were placed over the sunflower row covering the part between two rows. This base of the chamber was placed at a depth of 25 cm into the soil. The gas samples were collected between 07:00 and 10:00 following N fertilization events. The frequency of gas sampling was determined according to the fertilization time, and was once every 10 days in July, and once every 15 days in June, August, and September, and once per month in April, May, and October. The sample was not collected in May 2016. The irrigation water used for salt washing before planting needs to infiltrate for about one month in May 2016. In this period, we could not enter the field to collect gas. About 100 mL of gas was drawn through a 100-mL

injector connected to three sampling ports that passed through the chamber. The sampling time was 20 min per chamber. Samples were taken over 0, 5, 10, 15, and 20 min, with five samples per chamber and three replicate sets of samples per sampling. The collected gas samples, in cap-lock syringes, were quickly taken back to the laboratory, where they were analyzed using an Agilent 6820 gas chromatograph (Agilent 6820D, Agilent Technologies, Santa Clara, CA, USA) with a flame ionization detector. The flame ionization detector is used for the determination of N_2O and CH_4 . The emission was determined from the slope of the mixing ratio change in the five samples taken over a 20 min sampling period. The soil CH_4 uptake or N_2O emissions rate was estimated based on this regression. Sample sets that did not yield a linear regression value of r^2 greater than 0.90 were rejected. Rates of CH_4 uptake or N_2O emissions were determined from an average of three replicates under fertilized treatments and treatments without fertilization. The CH_4 or N_2O fluxes per unit area were calculated from Yang et al. (2018) [16].

2.3. Soil Collection and Analysis

The soil samples were extracted using a soil drill with a diameter of 0.05 m, using the S-shaped sampling method when collecting gas. The depth of this sampling was 0–20 cm. The sampling location was closed to the chamber. The samples were taken from 10 points per plot and mixed, then packed into sealed bags and brought back to the laboratory.

In the laboratory, the methods used for determining the soil organic carbon (SOC), total nitrogen (TN), soil $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, pH, and EC were as per the protocols of Yang et al. (2018) [16]. Physicochemical properties for S1 and S2 soils (Table 3) were determined according to the Chinese Soil Society guidelines for soil analysis. The concentrations of Cl^- , SO_4^{2-} , Na^+ , K^+ , Mg^{2+} , and Ca^{2+} were determined using a DIONEX ICS-3000 Ion Chromatography System [17]. Direct spectrophotometric measurements of (CO_3^{2-}) were performed using procedures outlined by Easley et al. (2013) [18]. Bicarbonate ion (HCO_3^-) content was determined using potentiometric titration [19]. The soil temperature and water content were measured in situ at a depth of 0.075 m using a temperature analyzer and TDR moisture analyzer, respectively.

2.4. Data Processing and Plotting

The differences and correlation analysis of CH_4 and N_2O fluxes from different nitrogen fertilization–saline soil were evaluated for statistical significance using a two-way analysis of variance (ANOVA) in Excel 2018. The random factors were considered in different growing seasons. The test data were processed and plotted using OriginPro 2021.

3. Results

3.1. Effects of Temperature and Moisture in the Soil on Seasonal Variation in CH_4 and N_2O Fluxes

The soil moisture content and temperature in S1 and S2 showed the same seasonal trends as the CH_4 uptake and N_2O emission. In July–August 2015–2016, S1 and S2 were under high temperature and moisture in the soil, and CH_4 uptake and N_2O emission fluxes were high. In contrast, these fluxes were relatively low in April–June and August–November. (Figures 2 and 3).

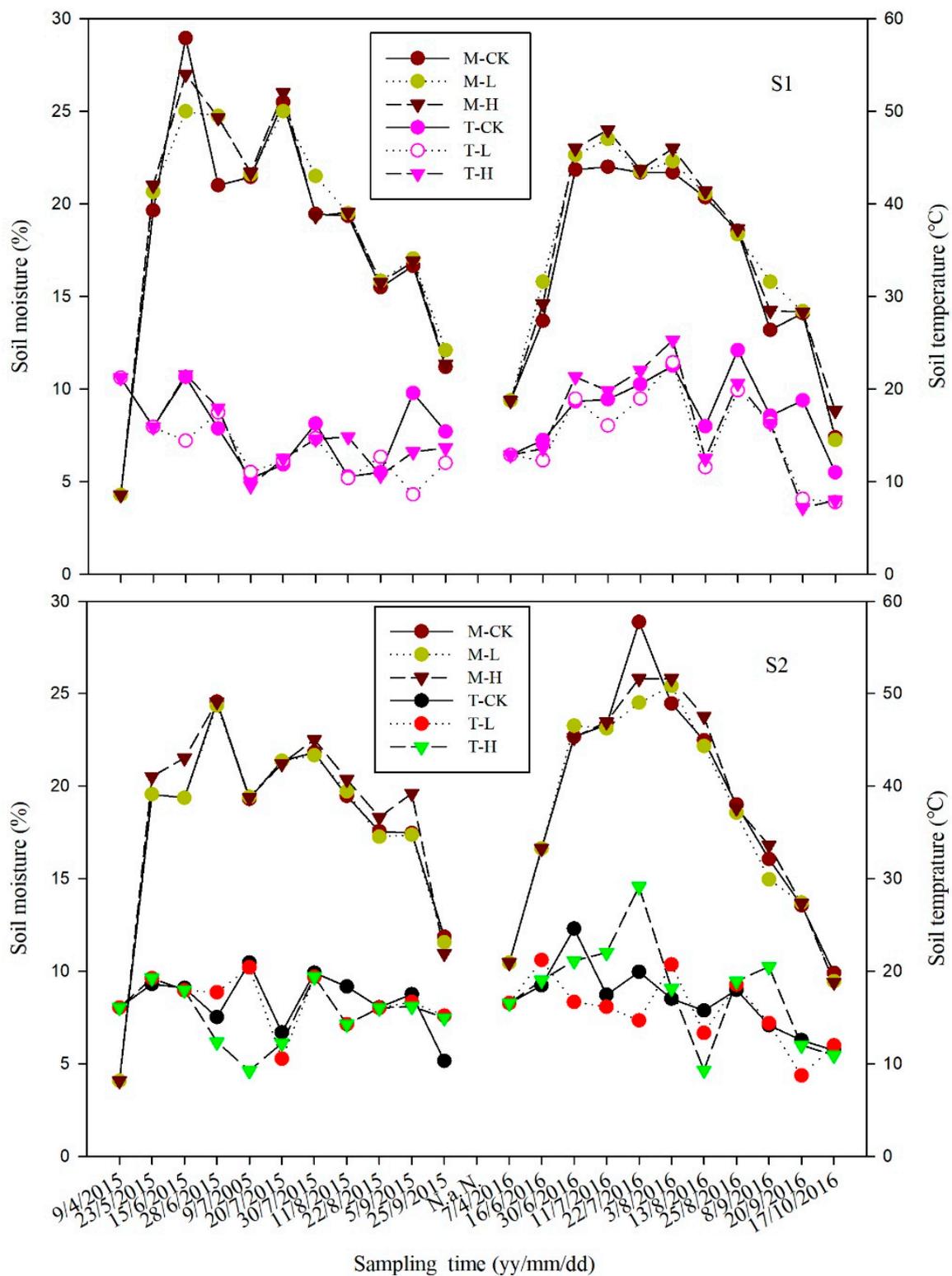


Figure 2. Variation pattern of soil moisture and soil temperature in the S_1 and S_2 with three levels of nitrogen fertilization (H, L, and CK) in saline soil from 2015 to 2016. (S_1 : slightly saline soil; S_2 : strongly saline soil; M: soil moisture; T: soil temperature).

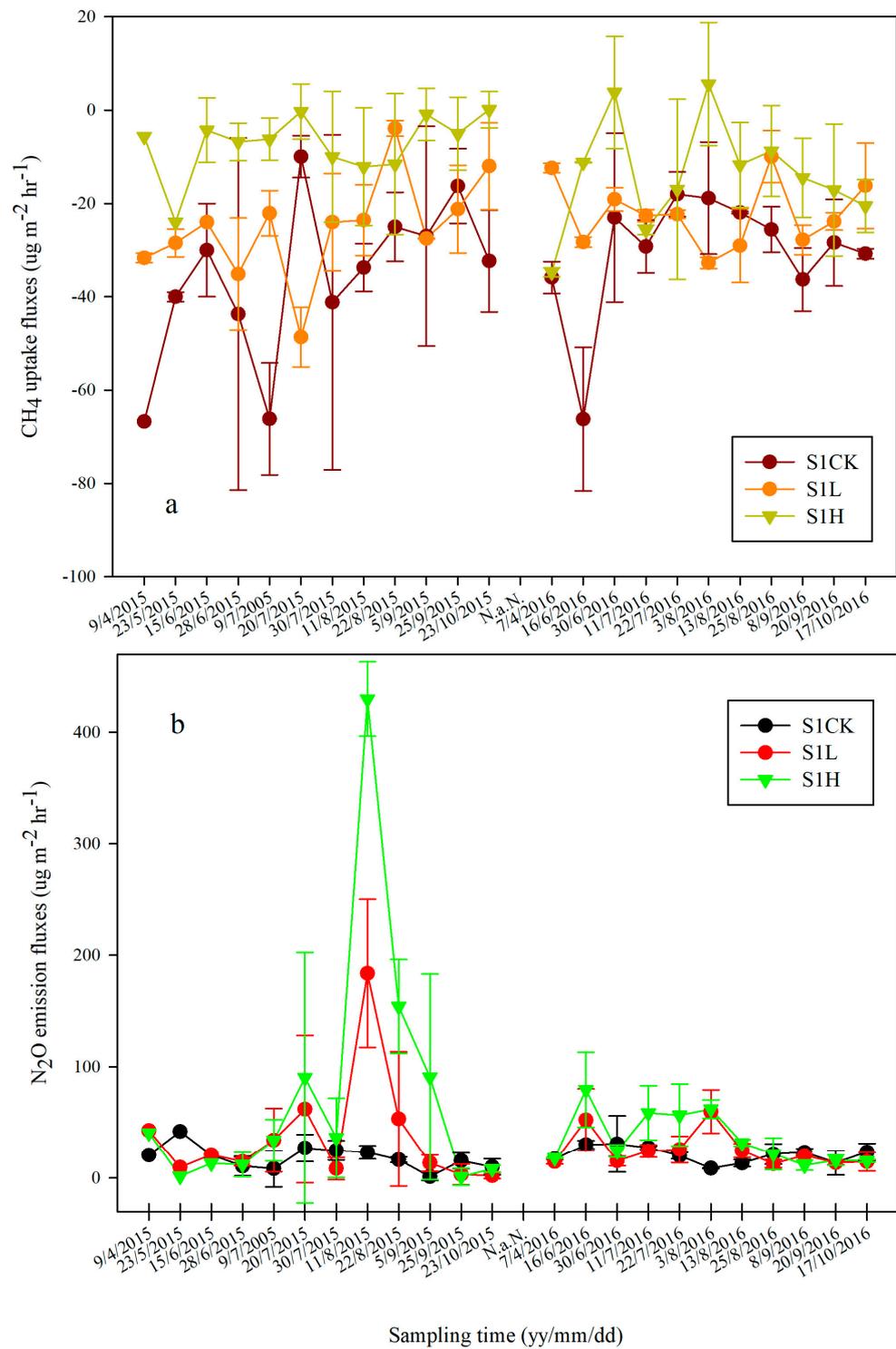


Figure 3. Cont.

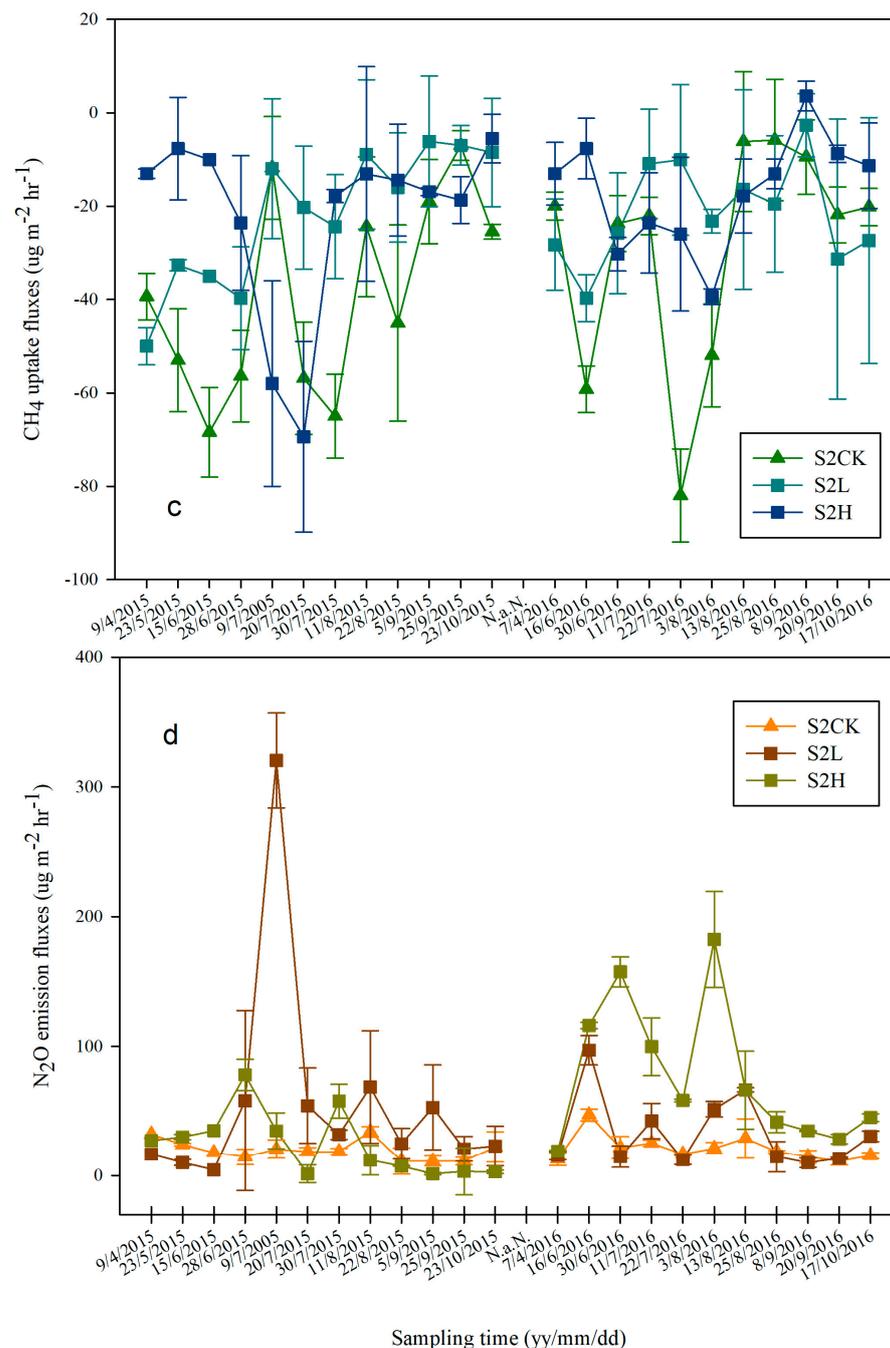


Figure 3. Variation pattern of CH₄ uptake fluxes (a) and N₂O emission fluxes (b) from S₁, and CH₄ uptake fluxes (c) and N₂O emission fluxes (d) from S₂ with three levels of nitrogen fertilization (H, L, and CK) in saline soil from 2015 to 2016. Vertical bars indicate the mean and standard error. (S₁: slightly saline soil; S₂: strongly saline soil).

The S₁ and S₂ soil exhibited the same trend of CH₄ and N₂O fluxes at different nitrogen fertilization rates during the whole crop growing season from 2015 to 2016. The CH₄ uptake flux peaked at 82.0 $\mu\text{g m}^{-2} \text{hr}^{-1}$ in July 2016, and the N₂O emission flux peaked at 429.8 $\mu\text{g m}^{-2} \text{hr}^{-1}$ in August 2015 (Figure 3). The H treatment lowered the CH₄ uptake fluxes, and increased N₂O emission fluxes, compared with the L treatment under the same salinity during the same growing season in 2015 and 2016. S₂ soil exhibited lower CH₄ uptake fluxes and higher N₂O emission fluxes than S₁ soil at the same nitrogen application rate in 2015 and 2016.

3.2. Cumulative N_2O Emission and CH_4 Uptake

The cumulative N_2O emission in the saline soils differed significantly between the two nitrogen fertilization rates during growing seasons in 2015 and 2016 ($p < 0.01$). The H treatment led to higher cumulative N_2O emission in saline soils than the L treatment under the same salinity in both growing seasons ($p < 0.01$). The cumulative N_2O emission augmented significantly with increasing salinity in saline soil at the same nitrogen application rate. The cumulative N_2O emissions in S1 under the CK, L, and H treatments were 97.0–99.4, 118.2–144.9, and 168.8–278.3 $mg\ m^{-2}$, respectively (Figure 4a). The cumulative N_2O emissions in S2 under the L and H treatments were 44.1–44.7%, and 74.1–91.1% higher than that in S1 in 2015 and 2016 ($p < 0.01$). The L and H treatments in S1 increased the cumulative N_2O emission by 18.9–49.4% and 69.8–186.9% in comparison to the CK treatment, respectively ($p < 0.01$). The cumulative N_2O emissions in S2 under the L and H treatments were 67.5–124.7% and 216.0–419.2% higher than that under the CK treatment, respectively (Figure 4a).

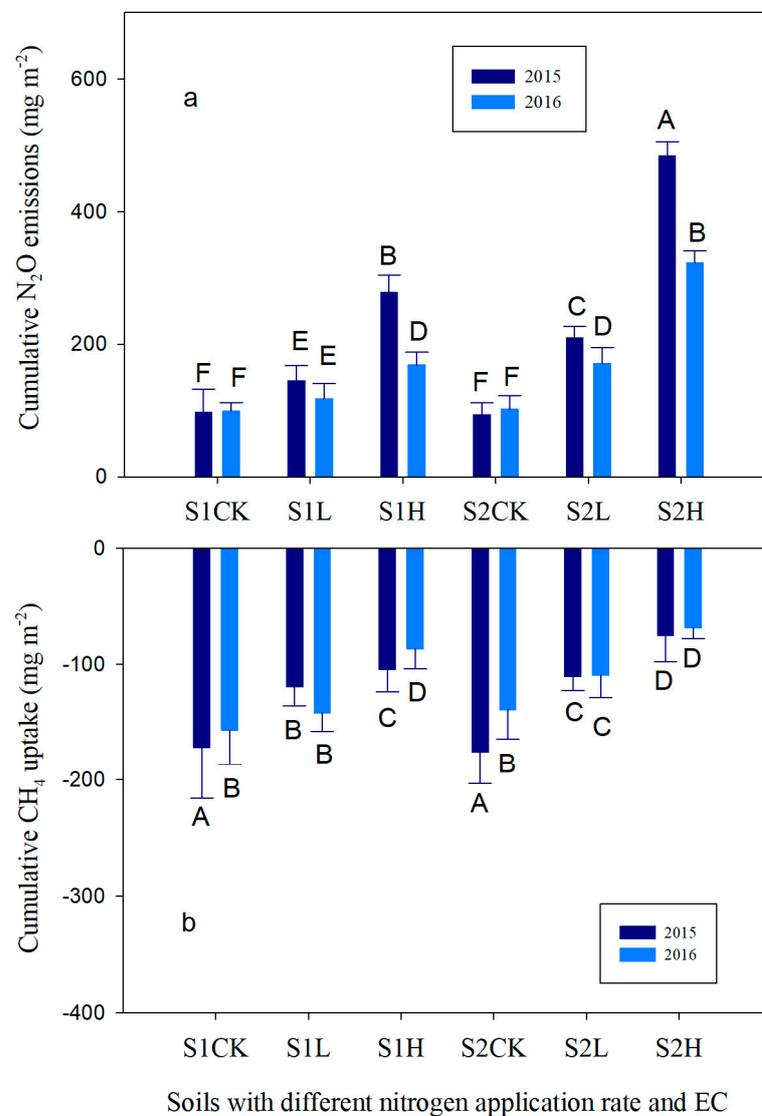


Figure 4. Cumulative N_2O emissions (a) and cumulative CH_4 uptake (b) in the S₁ and S₂ with three levels of nitrogen fertilization (H, L, and CK) in saline soil from 2015 to 2016. Vertical bars indicate the mean and standard error. There was significant difference for different letters (A–F) ($p < 0.01$). (S₁: slightly saline soil; S₂: strongly saline soil).

The cumulative CH₄ uptake ($F = 8.64$, $p < 0.01$) in S1 and S2 soils differed significantly between fertilizer treatments in the crop growing seasons from 2015 to 2016 (Figure 4b). The cumulative CH₄ uptake was higher in S1 than that in S2 for the CK, L, and H treatments ($p < 0.01$), and decreased with increasing salinity in saline soil. Low and high nitrogen fertilization rates inhibited CH₄ uptake in S1 and S2. Compared with the CK treatment, nitrogen application decreased CH₄ uptake in saline soils under the same salinity conditions ($p < 0.01$). The CK, L, and H treatments exhibited cumulative CH₄ uptake of 156.8–171.9, 119.7–142.0, and 86.7–104.8 mg m⁻² in S1, 139.3–176.0, 109.6–110.6, and 68.5–75.4 mg m⁻² in S2, respectively, during the growing season from 2015 to 2016. The cumulative CH₄ uptake of L treatments in S1 and S2 are 9.4–30.4% and 21.3–37.2% lower than that of CK treatment, respectively. The cumulative CH₄ uptake of H treatments in S1 and S2 are 39.0–44.7% and 50.8–57.2% lower than that of CK treatment, respectively ($p < 0.01$) (Figure 4b). During the growing seasons, the cumulative CH₄ uptake in saline soils for H treatment decreased considerably, compared with L treatment under the same salinity ($p < 0.01$). There was no significant difference in the cumulative CH₄ uptake under any treatment during growing seasons between 2015 and 2016 ($p > 0.05$).

The soil inorganic N content increased with increasing nitrogen application rate under the same salinity conditions (Table 4). Under the two salinity and three nitrogen application conditions, the cumulative N₂O emission showed a significant positive correlation with soil NO₃⁻-N content ($r = 0.8123$, $p < 0.01$), and EC ($r = 0.6403$, $p < 0.05$) (Figure 5).

Table 4. The chemical properties of different saline and fertilized soils.

Time	Treatments	NH ₄ ⁺ -N (mg kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)	Inorganic Nitrogen (mg kg ⁻¹)	pH
2015	S1CK	2.0 ± 0.18 a	33.0 ± 1.91 b	35.1 ± 1.81 b	8.0 ± 0.11 a
	S1L	4.1 ± 0.26 b	82.8 ± 4.20 c	86.9 ± 4.46 d	8.1 ± 0.09 a
	S1H	6.6 ± 0.07 c	150.5 ± 19.53 d	157.2 ± 19.60 e	8.2 ± 0.06 a
	S2CK	4.4 ± 0.96 b	19.4 ± 0.98 a	23.8 ± 1.94 a	8.7 ± 0.07 b
	S2L	6.6 ± 0.10 c	75.0 ± 3.34 c	81.6 ± 3.44 d	8.8 ± 0.12 b
	S2H	7.6 ± 0.16 d	125.0 ± 12.21 d	132.7 ± 12.37 e	8.8 ± 0.09 b
2016	S1CK	7.0 ± 0.13 d	16.2 ± 2.85 a	23.2 ± 2.98 a	8.1 ± 0.14 a
	S1L	9.5 ± 0.21 e	33.3 ± 4.56 b	42.7 ± 4.77 b	8.2 ± 0.11 a
	S1H	10.0 ± 0.32 e	41.5 ± 0.78 b	51.5 ± 1.01 c	8.1 ± 0.10 a
	S2CK	8.0 ± 0.19 d	14.1 ± 1.59 a	22.2 ± 1.78 a	8.7 ± 0.96 b
	S2L	10.2 ± 0.35 e	21.2 ± 0.98 a	31.4 ± 1.33 b	8.7 ± 0.93 b
	S2H	14.0 ± 0.39 f	30.7 ± 1.32 b	44.7 ± 1.71 b	8.8 ± 0.81 b

Note: S1, S2: slightly saline soil, and strongly saline soil. S1CK, S2CK: no fertilization of S1 and S2. S1L, S2L low-rate fertilization (175 kg N ha⁻¹) of S1 and S2. S1H, S2H: high-rate fertilization (350 kg N ha⁻¹) of S1 and S2. There was significant difference for different lowercase letters in the same column ($p < 0.05$). EC: Electrical conductivity; NH₄⁺-N: Ammonium; NO₃⁻-N: Nitrate. Values are means ± SD (n = 3).

However, neither the soil NH₄⁺-N nor its NO₃⁻-N content had a significant effect on CH₄ uptake ($p > 0.05$).

The effect of saline content, nitrogen application, and the two-factor interaction effects on the cumulative CH₄ uptake and N₂O emission in saline soil all reached a very significant level ($p < 0.01$). Nitrogen application was the important factor affecting N₂O emissions and CH₄ uptake in saline soil (Table 5). The F value shows the influence of nitrogen application and saline soil and its interaction on the N₂O emission in the following order: nitrogen application > saline soil > nitrogen application * saline soil; and on CH₄ uptake as nitrogen application * saline soil > saline soil > nitrogen application.

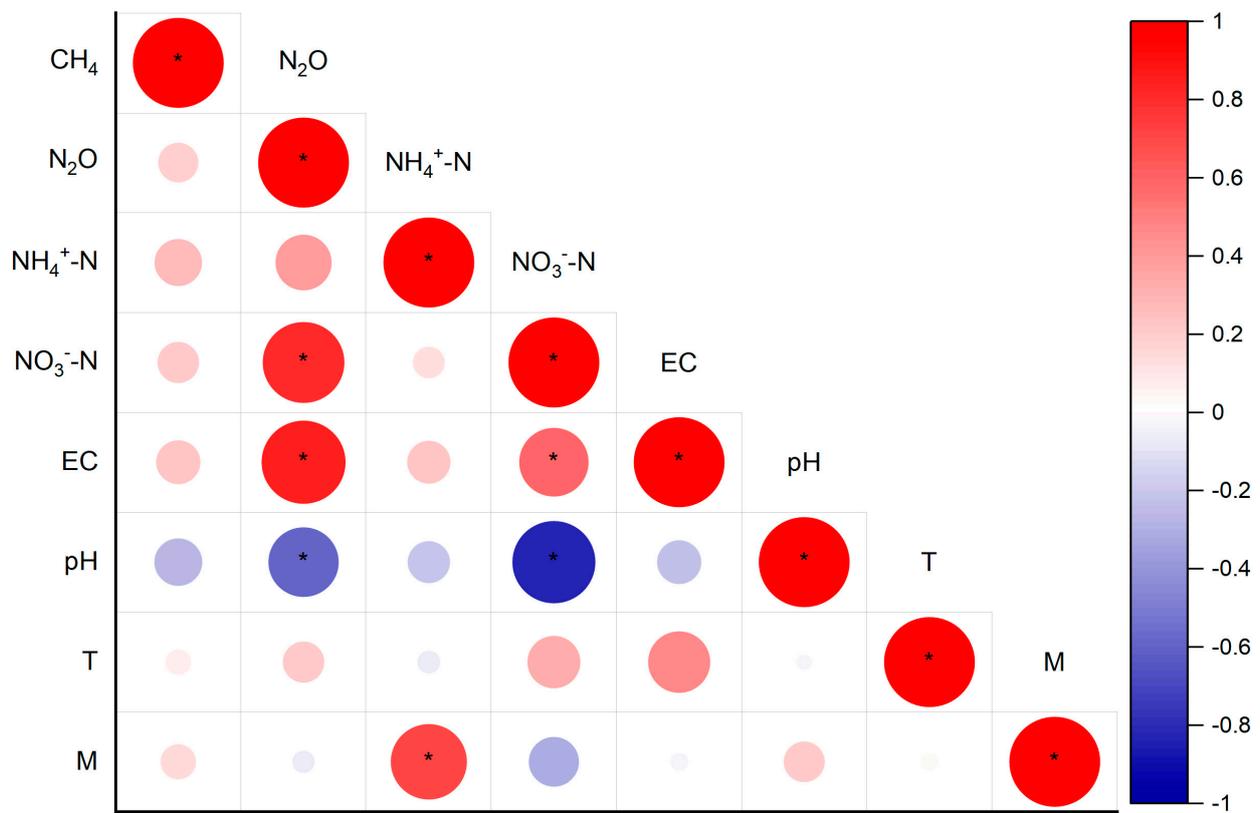


Figure 5. Pearson correlation analysis of CH₄ uptake and N₂O emissions (n = 18, * p ≤ 0.05). EC: Electrical conductivity; NH₄⁺-N: Ammonium; NO₃⁻-N: Nitrate; T: Soil temperature; M: Soil moisture.

Table 5. ANOVA for cumulative N₂O and CH₄ from the interaction with different nitrogen fertilization–saline soil.

Factors	Cumulative N ₂ O Amount			Cumulative CH ₄ Amount		
	F	p	Significance	F	p	Significance
Nitrogen fertilization	14.572	0.005	***	2.18	0.05	*
Saline soil	5.610	0.05	*	5.515	0.05	*
Nitrogen fertilization * saline soil	2.508	0.05	*	28.163	0.001	***

Note: * significance level at p < 0.05; *** significance level at p < 0.001.

4. Discussion

4.1. Fertilization Reduced CH₄ Uptake in Soils under Different Salinity Conditions

In our study, the cumulative CH₄ uptake of S1 was higher than that of S2 in both growing seasons, and the rate of the cumulative CH₄ uptake decreased with increasing soil salinity. Studies have reported that salinity inhibits CH₄ oxidation [20], and high salt content strongly inhibits CH₄ uptake [21]. Moderately saline soils have a higher potential for CH₄ uptake than strongly saline soils [22]. In addition, methanotrophs play an important role in the oxidization of atmospheric CH₄, and their activity directly affects CH₄ fluxes and the rate of CH₄ oxidation from soil to atmosphere [23,24]. The salt content in soil is the most important factor affecting microbial activity and can be correlated to the structure of the methanotrophs community (including species, abundance, diversity, and specific activity) [25]. CH₄ uptake was inhibited in strongly saline soil, likely because *Methylocella* spp., the most abundant methanotrophs, had low activity, whereas few were even unable to oxidize methane in strongly saline soils [26].

This study showed that nitrogen fertilization (diammonium phosphate and urea) application reduced CH₄ uptake in salinity conditions, consistent with previous reports. Grassmann et al. (2020) observed a decrease in CH₄ uptake because of the fertilization of grassland soils [27]. Powlson et al. (1997) used urea as a nitrogen fertilizer to study the methane-oxidizing ability of farmland soils, finding that long-term fertilization led to a significant decrease in the methane oxidation rate, and urea application inhibited soil methane oxidation by more than 80% in the short term, and the inhibition may be as high as 20%, after soil nitrification [28], since fertilization reduces the activity of methanotrophs in soils [29]. In addition, the effects of the non-specific salt from urea-derived NH₄⁺ inhibit soil CH₄ oxidation, thereby reducing soil CH₄ uptake [30]. This result was consistent with our research. The NH₄⁺-N content of saline soils in the H treatment was higher than that in the L treatment (Table 4). The effect of the nitrogen application rate on the CH₄ uptake by saline soils varied with soil salinity, compared with CK. CH₄ uptake in the S1 with the H and L nitrogen fertilization was lower than that of CK. CH₄ uptake was lower in the H treatment than in the L treatment. The minimum CH₄ uptake occurred at the highest fertilization rate of S2. Different amounts of nitrogen fertilizer (diammonium phosphate) applied to various saline soils had diverse effects on CH₄ fluxes, possibly because soil properties such as pH and salinity are key factors regulating bacterial diversity and community structure [31]. Soil temperature and moisture are not important single factors affecting CH₄ uptake. This may be because CH₄ uptake is subject to the cumulative effect of EC, moisture, and temperature; therefore, the contribution of soil temperature and moisture to CH₄ uptake is smaller and was obscured by the effects of nitrogenous fertilizer and EC to CH₄ uptake [16]. In the future, it will be necessary to study the effects of the activity and community structure of methanotrophs, and the methane oxidation rate in saline soils enhancing CH₄ uptake under the performance of agricultural and fertilization management practices. This could be an important research direction for gaining deeper insights into the effects and mechanisms of agricultural practices on CH₄ uptake in arable saline soils.

4.2. Effect of Nitrogen Fertilization on N₂O Emission in Saline Soils under Different Salinity Conditions

Indoor incubation experiments and field tests have shown that saline soils produce more N₂O emissions than non-saline soils after NH₄⁺ fertilization [16,32,33] Ghosh et al. (2017). This study showed that diammonium phosphate and urea application in saline soils significantly increased N₂O emission with increasing salinity, consistent with the above findings. Zhou et al. (2017a) reported that salinity induced increases in N₂O emissions, which is attributed to salt having a stronger inhibitory effect on nitrite reductase in comparison with ammonia-oxidizing bacteria (AOB) [34]. The functional nitrite reductase nir gene can be used as a molecular marker for denitrifying bacteria. It exhibits two structural forms, the copper-containing nitrite reductase (Cu-nir) encoded by the *NirK* gene and the cytochrome cd1-containing nitrite reductase (cd1-nir) encoded by the *nirS* gene. The *NirK* gene is more widely distributed than the *nirS* gene and is more sensitive to environmental factors. Another possible reason for the promotional effect of soil salinity on N₂O emission is that salinity reduces N₂O reductase activity. The reduction of N₂O to N₂ is the last step in the denitrification process in specific denitrifying bacteria and is primarily determined by N₂O reductase activity, which is more sensitive to the external environment than other N-cycling enzymes. High soil salinity strongly inhibits N₂O reductase. Under denitrification-favoring, NO₃⁻ N rich, anaerobic conditions soil N₂O emission shows a positive response to increased salinity [35,36]. This study showed that N₂O emission from nitrogen-fertilized saline soils ranged from 209.6 to 484.4 mg m⁻², which was significantly higher than in other non-saline fertilized areas [37,38], which is attributed to the fact that soil salinity promotes soil N₂O emission. Moreover, the shallow groundwater in the Hetao Irrigation District facilitates soil N₂O emission.

Soil N₂O emission under different salinity conditions increased with increasing nitrogen fertilization. Nitrogen fertilization promotes N₂O emission from saline soils because of large quantities of nitrogen present as a substrate for soil nitrification and denitrification, thereby facilitating N₂O emission [39]. The inorganic nitrogen content of soil decreases with increasing salinity [40,41]. This study showed that the nitrogen availability in saline soils varied with salinity under different fertilization treatments. The inorganic nitrogen content in S2 under the CK, L, and H treatments was lower than that in S1, respectively. Therefore, the soil inorganic nitrogen content decreased with increasing soil salinity under the same fertilization conditions.

This study showed that N₂O emissions increased steadily as the soil temperature and moisture increased. N₂O emissions generally peaked when soil temperature and moisture peaked. The two processes of nitrification and denitrification that microorganisms participated in had a significant effect on N₂O emissions, which are mainly related to soil temperature and moisture [42]. Inorganic nitrogen content in the studied soil increased with increasing nitrogen fertilization rate, whether the soil's salinity was high (S2) or low (S1). The content of NH₄⁺-N and NO₃⁻-N in soil are fundamental factors limiting soil N₂O production, whether through soil nitrification or denitrification. Nitrogen fertilization is favorable for the survival of nitrifying and denitrifying microorganisms in saline soils regardless of salinity level and can effectively reduce N₂O emission from nitrification, with the probability of N₂O formation through denitrification increasing with the increasing inorganic nitrogen content in soil [43]. This study revealed a highly significant positive correlation ($p < 0.01$) between N₂O emission and NO₃⁻-N content in saline soils (Figure 5). In the arable saline soils of the Hetao Irrigation District, N₂O is primarily produced by denitrification, as evidenced by that NO₃⁻-N has significant correlation with N₂O emission. The applied fertilizer provides substrate, nutrients, and a suitable environment for AOB and denitrifying bacteria (*NirK*) to facilitate soil denitrification and nitrifier denitrification. Further study should be conducted on the changes in the abundance of AOB and denitrifying bacteria (*NirK*) with increasing fertilization rates in saline soils with a fixed salinity level.

4.3. The Effects of Interactions from Fertilization and EC on the Cumulative CH₄ Uptake and N₂O Emissions

Nitrogen fertilization affects CH₄ and N₂O fluxes in saline soils. The results of N₂O emission and CH₄ uptake from fertilized saline soil in the experimental area were used to estimate the total greenhouse gas emissions in the region. The area of Hetao Plain affected by saline was 79.73×10^4 ha in Inner Mongolia, China [42]. The two-year mean cumulative N₂O emission from S1 in Hetao Plain of China over the growing season (from April to October) was estimated to be 0.78×10^6 kg, 1.05×10^6 kg, and 1.78×10^6 kg under the CK, L, and H treatments, respectively, and the cumulative CH₄ uptake was estimated to be 1.31×10^6 kg, 0.57×10^6 kg, and 1.04×10^6 kg, respectively. The corresponding results in S2 were 0.78×10^6 kg, 1.52×10^6 kg, and 3.22×10^6 kg for cumulative N₂O emission, and 0.88×10^6 kg, 1.26×10^6 kg, and 0.76×10^6 kg for cumulative CH₄ uptake, respectively. The cumulative N₂O emission in S1 and S2 from Hetao Plain account for 0.36–0.83% and 0.36–1.5% of the annual estimated N₂O emission of 2.15×10^9 kg in China [44], respectively. The cumulative CH₄ uptake in S1 and S2 from Hetao Plain under various fertilization conditions accounted for 0.21–0.47% and 0.27–0.45% of the annual estimated CH₄ uptake of 2.78×10^9 kg in China [45], respectively. The proportions of CH₄ uptake and N₂O emission in Hetao Plain for China approximated the proportion of CH₄ uptake and N₂O emission from saline soil in the Gurbantunggut Desert, Xinjiang, accounting for 0.23% and 0.52% of the national estimated value of China, respectively [46].

The slightly saline soils involved in CH₄ uptake could be considered a sink, but the capacity as a carbon sink decreased with increasing salinity and nitrogen fertilization in saline soil. Nitrogen fertilization increased the N₂O emissions in saline soils, and the N₂O emissions were significantly high in strongly saline soils at a high nitrogen fertilization rate. The CH₄ uptake and the N₂O emissions under different soil and fertilization conditions have shown that salt control and reduction of fertilizer application are effective measures to reduce the N₂O emissions and increase the CH₄ uptake in arable saline soils.

5. Conclusions

In this study, a two-year field experiment was performed to investigate the effects of nitrogen application level on the CH₄ uptake and N₂O emissions from intensively managed saline fields with different EC. The fluxes of CH₄ uptake and N₂O emissions from different nitrogen application and EC level varied remarkably in the growing seasons. Low salinity in the soil significantly inhibited N₂O emission and increased CH₄ uptake compared to soils with high salinity. CH₄ uptake decreased with increasing salinity and nitrogen application rates in saline soil. N₂O emission was promoted by nitrogen fertilization with increased salinity in saline soil. In terms of CH₄ uptake and N₂O emissions, treatment L would be recommended for soil S1. As a suggestion for future research, remediation of saline soil and optimization of fertilization are effective measures to reduce greenhouse gas emissions.

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