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Effects of Freezing–Thawing Processes on Net Nitrogen Mineralization in Salinized Farmland Soil

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Abstract: Nitrogen is an indispensable and limiting element for plant and microbial growth. To investigate the combined effects of salinity and freezing–thawing (FT) processes on soil inorganic nitrogen (SIN) transformation in seasonally freezing salinized farmland, laboratory incubation experiments were conducted under five soil salt content (SSC) treatments (0.08%, 0.25%, 0.35%, 0.50%, and 0.70%), four FT temperature treatments (C (5 °C), FT (−5 + 5 °C), FT (−10 + 5 °C), and FT (−15 + 5 °C)), and two soil water content (SWC) treatments (40% and 80% of maximum water holding capacity (WHC)). Ammonium (NH_4^+-N) and nitrate (NO_3^--N) nitrogen were monitored at the first, second, fifth, and eighth incubation days. The FT processes increased relative NH_4^+-N content by 13%, 39%, and 77% with the decreasing of freezing temperature from −5 °C to −15 °C compared with C (5 °C) treatments, respectively. FT (−5 + 5 °C) and FT (−15 + 5 °C) treatments decreased the relative NO_3^--N contents by 4% and 6% compared with C (5 °C) treatments, respectively. Under FT treatments, the increment of relative NH_4^+-N content was higher in low-SSC treatments and lower in high-SSC treatments. The relationship between relative NO_3^--N content and SSC gradually changed from a decrease in C (5 °C) to an increase in FT (−15 + 5 °C) treatments. SWC decreased NH_4^+-N content in high-SSC and low-freezing temperature treatments (SSC × freezing temperature < −2.5% °C), while NH_4^+-N increased in low-SSC and unfrozen treatments. The variations of SIN/R_{min} (nitrogen mineralization rate) were mostly affected by NO_3^--N/R_{nit} (net nitrification rate) and NH_4^+-N/Ra (net ammonification rate) in C (5 °C) and FT treatments, respectively. Overall, the results suggested that enhanced salinity inhibited the effects of freezing temperature on NH_4^+-N and NO_3^--N formation, respectively. The increase in SWC weakened the NH_4^+-N formation induced by the decrease in freezing temperature, and this function increased with the increase in salinity.

Keywords: ammonium nitrogen; nitrate nitrogen; salinity; soil water content

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1. Introduction

Nitrogen is an indispensable and limiting element for plant and microbial growth [1]. In total, 107.74 million tonnes of nitrogen were applied for global agricultural use in 2019 [2], of which only 47% was converted into harvested products [3]. Unabsorbed nitrogen will be lost to the atmosphere and water in the form of nitrous oxide, ammonium nitrogen, or nitrate nitrogen, which may contribute to the greenhouse effect and water eutrophication [4–6]. In the inland areas of northern China, the climate is dry and rainfall is low, and there is a large area of salinized farmland due to poor irrigation management, insufficient drainage, improper cropping patterns and rotations, and chemical contamination [4,7,8]. These farmlands are affected by a continental monsoon climate, where winter and spring last for up to half a year, and the soil experiences an obvious seasonal freezing–thawing (FT) phenomenon [9,10]. Soil salinity and FT cycles (FTCs) are the main factors affecting soil nitrogen transformation in this region [11,12].

To date, a large number of studies have investigated the effect of soil salinity on nitrogen transformation [11,13,14]. Salinity mostly impacts N mineralization via osmotic potential and specific iron effects on microorganisms [4,15]. Higher salinity results in high osmotic potential, which may induce the drying and lysis of microbial cells [4,16]. The sensitivity of different nitrogen conversion processes to soil salinity varies significantly [17]. Salinity can increase N mineralization by enhancing the relative abundance and activity of the specific microbial functional groups responsible for N mineralization, or by inhibiting microbial N immobilization [1,14,15,18]. Nitrification will be promoted when the soil salinity is lower than 1.13 dS/m and will be inhibited when soil salinity is higher [19]. In addition, SO_4^{2-} exhibits a stronger inhibitory effect on nitrification than Cl^- [15]. Denitrification is easily inhibited by increased salinity because elevated salinity can induce sulfide toxicity to denitrifying bacteria, shift soil redox conditions, change the availability of C and N substrates, and reduce soil oxygen consumption [18–22]. Salinity is a dominant factor affecting the growth of ammonia-oxidizing microorganisms and community structure [23]. It increases the intensity and prolongs the duration of ammonia volatilization, inducing a higher amount of ammonium nitrogen loss [24–26].

The FT process is a key driver of soil nitrogen transformation in cold regions that affects soil mineral nitrogen content by changing soil physical structure, chemical properties, and biological properties [27–29]. Soil water freezes and forms ice crystals during the freezing process, which induces soil expansion and destroys the structure of soil aggregates [30]. The organic carbon and inorganic nitrogen that were originally wrapped and adsorbed are released to provide substrates, thereby increasing the subsequent mineralization [17,31]. In addition, the newly exposed mineral surfaces of organic–mineral complexes during this process can significantly affect the adsorption and desorption of nitrogen [29]. The freezing and thawing of soil water can also affect nitrification and denitrification by blocking soil porosity and changing the soil structure to create an alternating anaerobic and aerobic environment [32–34]. Extreme alternating changes in soil temperature and liquid water content in a short period of time (24 h) will greatly affect soil nitrogen transformation by changing the structure, quantity, diversity, and activity of soil microbial communities [33,35–37].

In seasonally freezing saline farmland, the soil FT process is accompanied by complex changes in moisture, salinity, and temperature [12,32,38]. For example, the temperature gradient in the freezing process and evaporation in the early thawing process will induce considerable salt accumulation in the surface layer, altering the salt distribution in the soil profile [39,40]. The presence of salt will influence the liquid water and ice crystal content in the soil during the freezing process, thus altering the soil structure and air permeability [41–43]. These changes will directly or indirectly affect the soil microbial community, quantity, diversity, and activity, thereby affecting the nitrogen transformation [37,44,45]. However, most of the existing studies only consider the effect of a single factor (salinity or FT) on soil nitrogen transformation, and few investigations of their interactive effects on nitrogen transformation have been performed.

Under ongoing global warming and given the increasing scarcity of water resources [46–49], salinized farmlands are facing more challenges, including the decreasing frequency of FTCs during winter and spring [49], water shortages for irrigation and leaching [5,50], and an increased risk of soil salinization [51,52]. The nitrogen cycle in these areas will become more complex, and the management of water and fertilizer will become more challenging. Therefore, in this study, a laboratory experiment was conducted to investigate the combined effects of salinity and FT processes on soil inorganic nitrogen transformation in seasonal freezing salinized farmland, and explore the influence mechanisms. The findings of this study have important theoretical and practical significance for accurately describing the nitrogen cycle in seasonal FT saline farmland systems, and provide a basis for improving water and fertilizer management in this region.

2. Materials and Methods

2.1. Study Site and Soil Sampling

The sampling site was located at the Yonglian experimental station in the Hetao Irrigation District of the Inner Mongolia Autonomous Region, China (108°37′ E, 40°15′ N). This area has a mid-temperate continental desert monsoon climate and has an annual average precipitation of 170.0 mm, an annual average temperature of 6.1 °C, and a frost-free period of 117–136 days.

Topsoil (0−10 cm depth) samples were randomly collected using a 10 cm auger in a corn field with low soil salinity (soil salt content (SSC) = 0.08%) in July 2021. After removing stones, plant debris, and large roots, each soil sample was air-dried and well-mixed in preparation for the determination of the soil properties and incubation experiments. The soil physicochemical properties are listed in Table 1.

Table 1. Soil properties for incubation experiment.

pH	EC (μS/cm)	TN (mg/kg)	TC (mg/kg)	C/N	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	Clay (%)	Slit (%)	Sand (%)	Soil Texture
8.33 ± 0.03	364.84 ± 14.34	0.82 ± 0.02	23.75 ± 0.59	28.96	8.30 ± 0.85	7.80 ± 0.55	6.79 ± 0.19	45.04 ± 0.48	48.17 ± 0.67	Silty loam

Note: EC, TN, TC, C/N, NH_4^+-N, and NO_3^--N denote electricity conductivity, total nitrogen, total carbon, ratio of total carbon to total nitrogen, ammonium nitrogen, and nitrate nitrogen, respectively.

2.2. Experimental Design

The experimental flow chart is shown in Figure 1. A completely random block design was conducted in the current study, with three factors: four FT temperature treatments (freezing for 12 h and thawing for 12 h: +5 °C + 5 °C, −5 + 5 °C, −10 + 5 °C, and −15 + 5 °C), five levels of SSC (0.08%, 0.25%, 0.35%, 0.50%, and 0.70%), and two soil water content (SWC) treatments (40% and 80% of water holding capacity (WHC)).

Soil samples were prepared by mixing air-dried soil, mixed salt solution, $CaCl_2$ solution, and deionized water in a certain proportion. The mixed salt solution was compounded by adding Na_2SO_4, $MgCl_2$, $NaHCO_3$, NaCl, and KCl to deionized water. The mass ratio of ions added to soil was determined by the local salinity of the soil, which was K^+: Na^+: Ca^+: Mg^+: Cl^-: HCO_3^-: SO_4^{2-} = 2.49%: 22.12%: 4.67%: 3.37%: 21.37%: 5.31%: 40.67%. After reaching the preset water and salinity contents, all soil samples were packed into a quartz glass column (inner diameter: 23 mm; height: 20 mm) with a soil bulk density of 1.4 g/cm^3 and a dry soil mass of 10 g. These glass columns were sealed with plastic film, and six holes were pinned at the top side for ventilation, and then transferred into the dark incubator for pre-incubation for 20 days at 20 °C and 60% relative humidity (RH). After pre-incubation, soil samples were divided into two portions. One portion was incubated in a temperature-controlled refrigerator at 5 °C as a control treatment (C (5 °C)). The remaining soils were incubated in a temperature-controlled FTC device at a freezing temperature of either −5 °C, −10 °C, or −15 °C for 12 h and then a thawing temperature of 5 °C for another 12 h; this was considered as one FTC. The incubation lasted for 8 days, and 120 soil samples were randomly collected before FTC incubation and after the first, second, fifth, and eighth FTC. In total, 600 samples (three replicates for each treatment in each of the five sampling periods) were incubated.

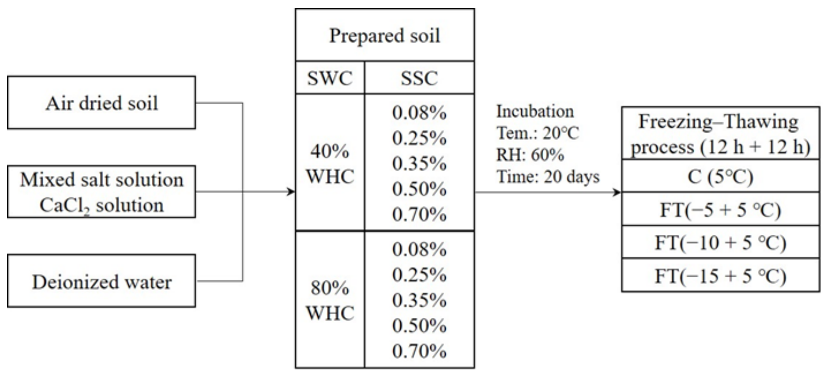


Figure 1. Experimental flow chart.

2.3. Sample Analysis

Soil total carbon (TC), total nitrogen (TN), particle gradation, electrical conductivity (EC), and pH were measured before FT incubation, and soil NH_4^+-N and NO_3^--N were measured for each treatment. Soil TC and TN were measured on an elemental analyzer (Vario Ei, Elementar Co., Hamburg, Germany). Soil particle gradation was measured by a particle size analyzer (S3500, Microtrac Inc., Montgomeryville, PA, USA). The EC and pH of soil extracts with a soil water ratio of 1:5 were determined by an EC probe and pH probe, respectively (DZS-706 Multi-parameter Analyzer, Hunan Lichen Instrument Technology, Changsha, China). Soil NH_4^+-N and NO_3^--N were analyzed using a CleverChem 200 automatic continuous analyzer (DeChem-Tech. GmbH, Hamburg, Germany) after extraction with 2 mol/L KCl solution in a soil-to-KCl solution ratio of 1:5.

2.4. Net Mineralization Rates

The net soil nitrogen mineralization rate (R_{min}) was calculated as the difference between the soil inorganic nitrogen (*SIN*) contents of two sampling periods divided by the incubation time. A similar formula was used to calculate the net N ammonification rate (R_a) and net nitrification rate (R_{nit}), as follows.

$$R_{min} = \frac{SIN\text{ content after incubation period } - SIN\text{ content before incubation period}}{\text{incubation days}} \quad (1)$$

$$R_a = \frac{NH_4{}^+ - N\text{ content after incubation period } - NH_4{}^+ - N\text{ content before incubation period}}{\text{incubation days}} \quad (2)$$

$$R_{nit} = \frac{NO_3{}^- - N\text{ content after incubation period } - NO_3{}^- - N\text{ content before incubation period}}{\text{incubation days}} \quad (3)$$

To evaluate the effects of salinity and moisture on inorganic nitrogen transformation during FT processes, the ratios of the time-weighted mean soil NH_4^+-N, NO_3^--N, and SIN content of each salinity and moisture treatment to their initial values before FT incubation were calculated using

$$R_N = \frac{(N_0 + N_1) \times 1 + (N_1 + N_2) \times 1 + (N_2 + N_5) \times 3 + (N_5 + N_8) \times 3}{2 \times N_0} \quad (4)$$

where N represented NH_4^+-N, NO_3^--N, and SIN, and 0, 1, 2, 5, and 8 designated the sampling days.

2.5. Statistical Analysis

Multi-way analysis of variance (ANOVA) was used to identify the effects of the salinity, freezing temperature, moisture, and incubation period and their interactions on the inorganic nitrogen (SIN, NH_4^+-N, and NO_3^--N) content and net transformation rate (R_{min}, R_a, and R_{nit}). All statistical analyses were performed using SPSS 13.0 software (SPSS, Chicago, IL, USA).

3. Results

3.1. Soil Inorganic N Variation under Different SSC, FT Temperatures/Cycles, and SWC

As shown in Table 2, after 20 days of pre-incubation, NH_4^+-N, SIN, and the value of NH_4^+-N/SIN decreased from 8.30 mg/kg, 16.10 mg/kg, and 51.55, respectively, to 1.09–1.57 mg/kg, 9.70–13.84 mg/kg, and 9.70–13.84, respectively, while NO_3^--N increased from 7.80 mg/kg to 8.51–12.61 mg/kg. The contents of TN, TC, and C/N remained essentially unchanged. The decrease in NH_4^+-N, the value of NH_4^+-N/SIN, and the increase in NO_3^--N was higher in the treatments with higher soil moisture (80% WHC).

Table 2. Soil NH_4^+-N, NO_3^--N, SIN, TN, and TC content, and NH_4^+-N/SIN and C/N after pre-incubation.

SWC	SSC (%)	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	SIN (mg/kg)	NH_4^+-N /SIN (%)	TN (mg/kg)	TC (mg/kg)	C/N
40 % WHC	0.08	1.57 ± 0.84 a	8.69 ± 2.10 b	10.26 ± 2.28 b	15.20	0.81 ± 0.03 a	23.50 ± 0.21 bcd	29.01
	0.25	1.55 ± 0.59 a	9.77 ± 2.86 ab	11.33 ± 2.52 ab	14.78	0.79 ± 0.03 a	23.26 ± 0.33 cd	29.44
	0.35	1.19 ± 0.42 a	8.51 ± 1.84 b	9.70 ± 1.83 b	12.70	0.81 ± 0.02 a	23.13 ± 0.37 d	28.73
	0.5	1.37 ± 0.79 a	9.67 ± 2.52 b	11.03 ± 2.28 ab	12.93	0.81 ± 0.01 a	23.49 ± 0.27 bcd	29.17
	0.7	1.56 ± 0.92 a	9.40 ± 0.80 b	10.96 ± 0.95 ab	13.84	0.79 ± 0.01 a	23.15 ± 0.09 cd	29.48
80% WHC	0.08	1.23 ± 0.51 a	12.61 ± 1.41 a	13.84 ± 1.67 a	8.74	0.80 ± 0.06 a	24.27 ± 0.03 ab	30.53
	0.25	1.15 ± 0.41 a	11.20 ± 1.15 ab	12.35 ± 1.35 ab	9.24	0.81 ± 0.01 a	26.15 ± 2.22 a	32.28
	0.35	1.09 ± 0.29 a	12.19 ± 0.99 a	13.29 ± 1.01 a	8.25	0.82 ± 0.02 a	24.39 ± 0.15 a	29.92
	0.5	1.21 ± 0.45 a	11.18 ± 1.02 ab	12.39 ± 1.45 ab	9.49	0.83 ± 0.01 a	24.43 ± 0.22 a	29.43
	0.7	1.47 ± 0.48 a	12.23 ± 1.10 a	13.70 ± 1.02 a	10.79	0.83 ± 0.01 a	23.97 ± 0.11 abc	29.05

Note: SWC, SSC, NH_4^+-N, NO_3^--N, SIN, TN, TC, C/N, and WHC denote soil water content, soil salt content, ammonium nitrogen, nitrate nitrogen, soil inorganic nitrogen, total nitrogen, total carbon, ratio of total carbon to total nitrogen, and water holding capacity, respectively. a, b, c, and d indicate the significant difference in each nitrogen and carbon index among the treatments ($p < 0.05$).

During FT incubation, the variation trends of NH_4^+-N, NO_3^--N, and SIN were similar under both salinity and moisture treatments but changed significantly under FT temperature and cycling treatments (Figures 2 and A1).

Soil NH_4^+-N contents during the first 2 days of incubation decreased in the C (5 °C) treatment, increased and then decreased under the FT (−5 + 5 °C) and FT (−10 + 5 °C) treatments, and increased under the FT (−15 + 5 °C) treatment. During the following incubation days, soil NH_4^+-N contents mostly increased in the C (5 °C) treatment, while they decreased during incubation days 3–5 under the FT treatments, and decreased, remained almost unchanged, and mostly increased during incubation days 6–8 under the FT (−5 + 5 °C), FT (−10 + 5 °C), and FT (−15 + 5 °C) treatments, respectively. Freezing temperature, salinity, the interactions between salinity and water, and the interactions between salinity and the FTCs had significant effects on the variation of soil NH_4^+-N content (Table 3).

Soil NO_3^--N contents mostly increased during the first two incubation days under both the control and FT treatments, except for the FT (−15 + 5 °C) treatment, in which the soil NO_3^--N contents mostly decreased during the first day. During the following incubation days, soil NO_3^--N contents mostly decreased in the control treatment, decreased during days 3–5, and increased during days 6–8 under FT (−5 + 5 °C), while they increased under the FT (−10 + 5 °C) and FT (−15 + 5 °C) treatments. Salinity, as well as the interactions between salinity and moisture, salinity and FTCs, and among salinity, moisture, and FTCs, have significant effects on the variation of soil NO_3^--N content.

There was no clear variation trend of SIN in C (5 °C), but it showed an obvious rise followed by a falling trend during the first 2 days under the FT (−5 + 5 °C) and FT (−10 + 5 °C) treatments, and a fall followed by a rise or a stable rise trend under the FT (−15 + 5 °C) treatment. During the following days, the SIN content increased stably under the FT (−10 + 5 °C) treatment, while it mostly decreased during incubation days 3–5 and increased during incubation days 6–8 under the FT (−5 + 5 °C) and FT (−15 + 5 °C) treatments. Soil salinity, moisture, FTCs, the interactions between salinity and moisture, salinity and FTCs, moisture and freezing cycles, and the interactions among salinity, moisture, and FTCs significantly affected the variation of SIN content.

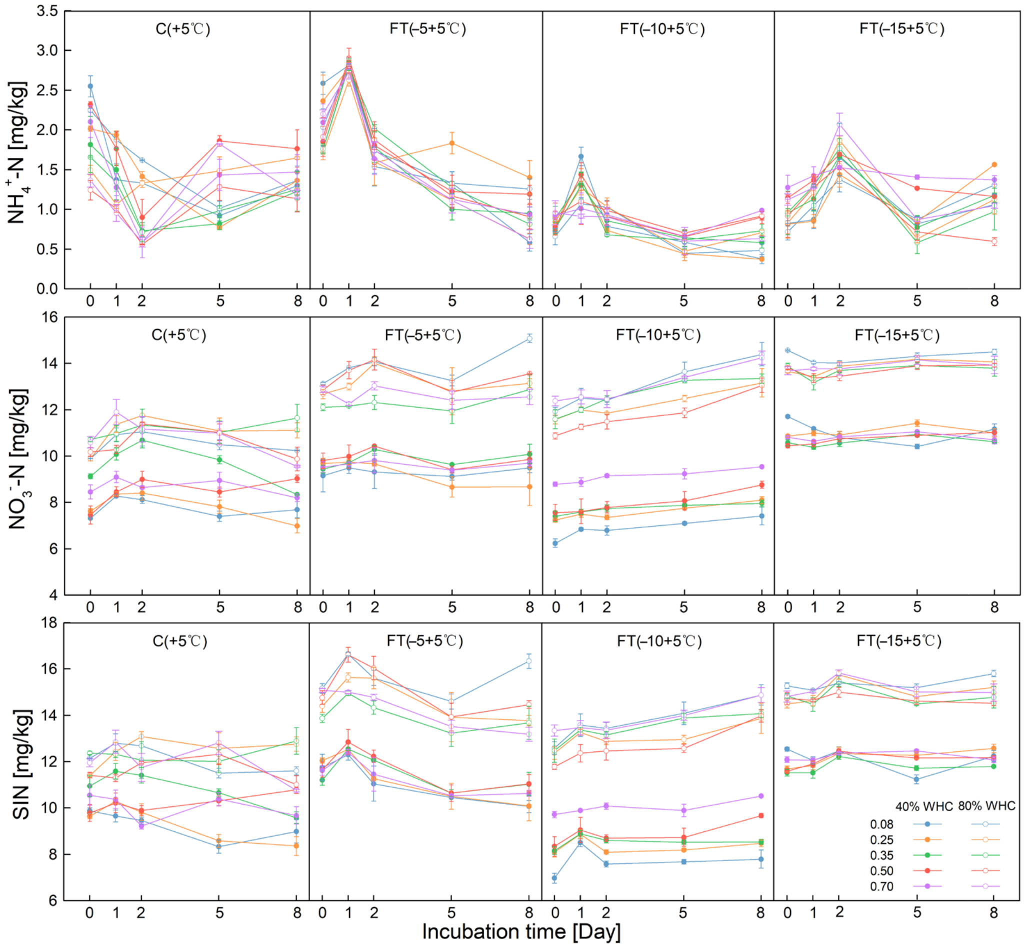


Figure 2. Soil NH_4^+-N, NO_3^--N, and soil inorganic nitrogen (SIN) changes during the incubation period under different salinity, freezing–thawing (FT) temperatures/cycles, and soil moisture contents.

Table 3. Results of multi-way analysis of variance (ANOVA) on soil mineral N variation under different incubation freezing–thawing temperature/cycles, SSC, and SWC (T: temperature; SSC: soil salt content; SWC: soil water content; FTC: number of freezing–thawing cycles; SIN: soil inorganic nitrogen).

Factors	NH_4^+-N		NO_3^--N		SIN	
	F	Sig.	*F*	Sig.	*F*	Sig.
T	3.830	0.010 *	0.133	0.941	1.771	0.153
SSC	31.019	0.000 **	23.837	0.000 **	12.964	0.000 **
SWC	3.024	0.083	0.396	0.529	4.249	0.040 *
FTC	1.815	0.144	2.345	0.073	6.408	0.000 **
T × SSC	1.097	0.362	1.433	0.149	0.920	0.527
T × SWC	0.078	0.972	0.676	0.568	0.907	0.438
T × FTC	0.156	0.998	0.374	0.947	0.472	0.893
SSC × SWC	4.040	0.003 *	22.577	0.000 **	23.520	0.000 **
SSC × FTC	2.876	0.001 *	5.191	0.000 **	7.843	0.000 **
SWC × FTC	1.723	0.162	3.391	0.018 *	2.882	0.036 *
T × SSC × SWC	0.466	0.933	0.567	0.868	0.493	0.918
T × SSC × FTC	0.248	1.000	0.415	0.999	0.326	1.000
T × SWC × FTC	0.255	0.986	0.806	0.611	0.510	0.867
SSC × SWC × FTC	1.178	0.298	7.681	0.000 **	4.932	0.000 **
T × SSC × SWC × FTC	0.173	1.000	0.578	0.976	0.39	0.999

**, $p < 0.01$; *, $p < 0.05$.

3.2. Soil Inorganic N Content Affected by SCC, FT Temperature, and SWC

The ratios of the time-weighted mean soil NH_4^+-N, NO_3^--N, and SIN content of each treatment to its initial value are shown in Figure 3. During the FT incubation, the increase in NH_4^+-N occurred when the freezing temperatures were −10 and −15 °C (Figure 3a,d). The relative contents of NH_4^+-N under FT (−5 + 5 °C), FT (−10 + 5 °C), and FT (−15 + 5 °C) treatments were 1.13, 1.39, and 1.77 times higher compared with that under C (5 °C) treatments. The relative contents of NH_4^+-N increased (Figure 3d) or increased and then decreased (Figure 3a) with the increase in salinity under the C (5 °C) treatments. In contrast, in the FT treatments, with the decrease in freezing temperature, the relative contents of NH_4^+-N gradually changed into a single downward trend with the increase in salinity. FT increased the NH_4^+-N increment under low salinity.

NO_3^--N content increased under most of the control and FT soil treatments (Figure 3b,e). The relative NO_3^--N contents under FT (−5 + 5 °C) and FT (−15 + 5 °C) treatments were 0.96 and 0.94 times lower compared with under C (5 °C) treatments but were 1.01 times higher under FT (−10 + 5 °C) treatments. The relative contents of NO_3^--N decreased with the increase in salinity in the C (5 °C), FT (−5 + 5 °C), and FT (−10 + 5 °C) treatments and the decreasing trend weakened with the reduction of freezing temperature. Finally, the trend shifted to an increasing trend with increasing salinity under the FT (−15 + 5 °C) treatment. The FT (−5 + 5 °C) and FT (−10 + 5 °C) treatments reduced the NO_3^--N content and the reduction was higher under the low salinity treatment.

The SIN content increased under the FT (−10 + 5 °C) and FT (−15 + 5 °C) treatments and mostly decreased under the C (5 °C) and FT (−5 + 5 °C) treatments (Figure 3c,f). The relative contents of SIN were significantly increased under the FT (−10 + 5 °C) × SWC (40% WHC), FT (−15 + 5 °C) × SWC (40% WHC), and FT (−10 + 5 °C) × SWC (80% WHC) treatments. Under the SWC (40% WHC) treatments, the relative contents of SIN increased and then decreased with the increase in salinity, except for in the FT (−10 + 5 °C) treatment, in which the relative content of SIN decreased with the increase in salinity. Under SWC (80% WHC) treatments, the relative contents of SIN decreased with the increase in salinity, except for in the C (5 °C) treatment, in which the relative content of SIN increased and then decreased with the increase in salinity.

The variation ranges and coefficients of variation of relative NH_4^+-N, NO_3^--N, and SIN contents induced by freezing temperature, salinity, and moisture are shown in Figure 4. Variations in relative NH_4^+-N, NO_3^--N, and SIN contents induced by freezing temperature reduced with the increase in salinity (Figure 4a,b,c), and the variation in relative NO_3^--N and SIN induced by salinity were greatly reduced with decreased freezing temperature (Figure 4d,e,f). Moreover, an increase in soil moisture reduced the variations in NH_4^+-N induced by freezing temperature (Figure 4a) as well as the variations in NO_3^--N and SIN induced by salinity (Figure 4e,f).

The differences in relative N content compared to its initial values between SWC (80% WHC) treatments and SWC (40% WHC) treatments are shown in Figure 4. The relative NH_4^+-N contents in SWC (80% WHC) treatments were lower than in SWC (40% WHC) treatments when they were exposed to a combination of low temperature and high salt (SSC × freezing temperature < −2.5% °C) (Figure 5). The relative NO_3^--N and SIN contents were higher in SWC (80% WHC) treatments when SSC was lower than 0.35%.

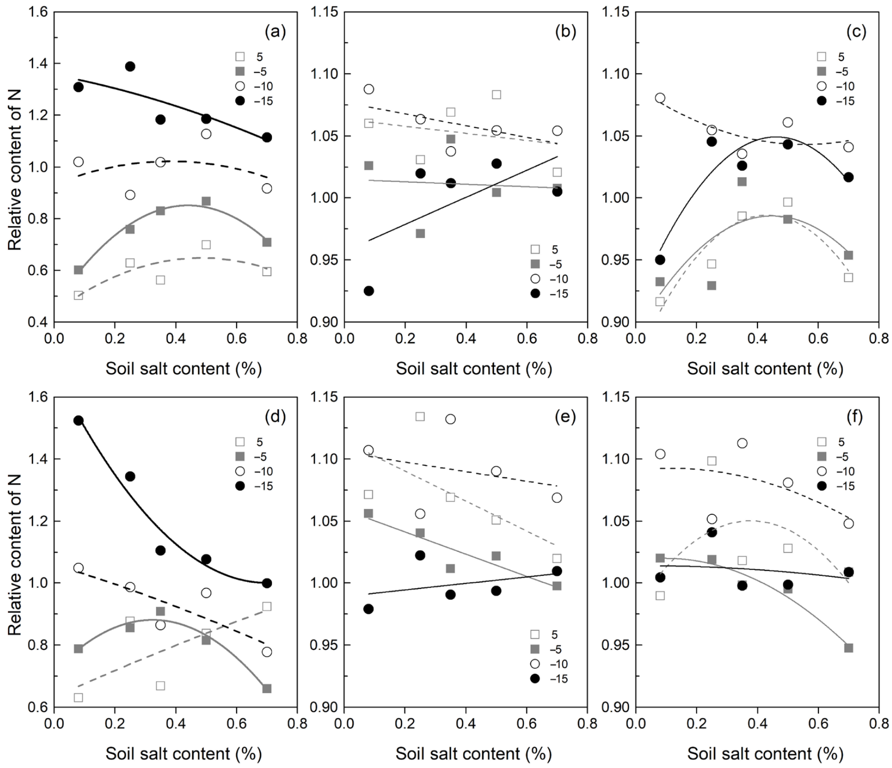


Figure 3. Ratio of the time-weighted mean soil NH_4^+-N ((**a**): 40% water holding capacity (WHC); (**d**): 80% WHC), NO_3^--N ((**b**): 40% WHC; (**e**): 80% WHC), and soil inorganic nitrogen (SIN) (**c**): 40% WHC; (**f**): 80% WHC) contents to their initial values before freezing–thawing incubation.

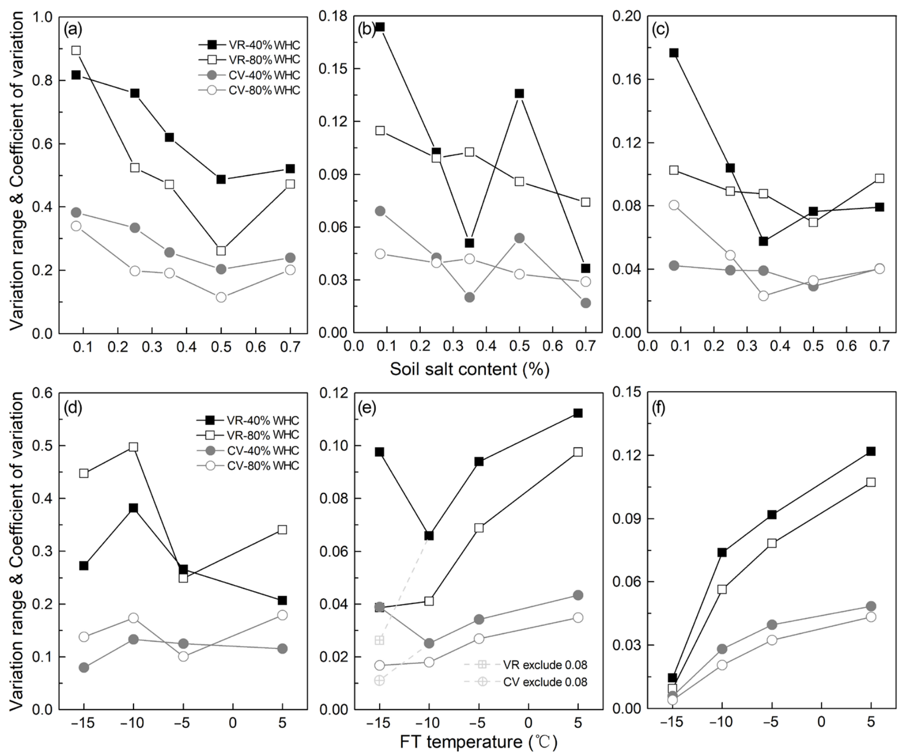


Figure 4. Variation ranges and coefficients of variation of relative soil N (NH_4^+-N, NO_3^--N and SIN) induced by different freezing temperatures in different soil salt content (SSC) and soil water content (SWC) treatments (**a**–**c**), and induced by SSC in different freezing–thawing (FT) and SWC treatments (**d**–f).

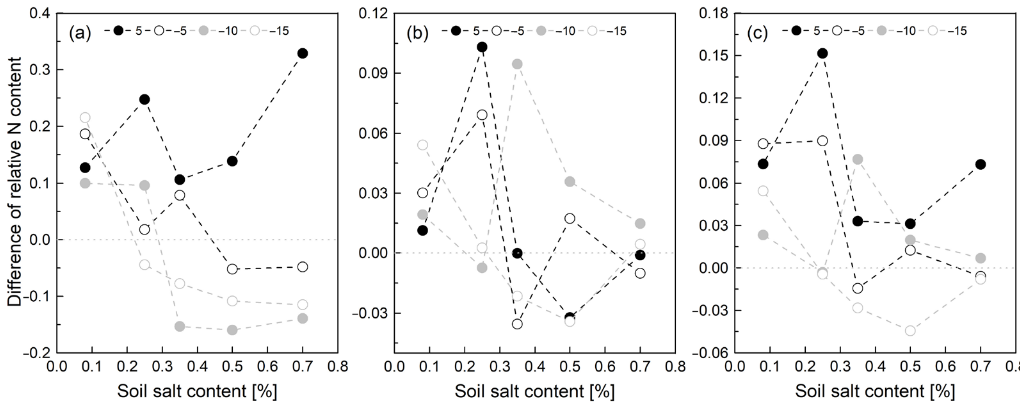


Figure 5. Differences in relative NH_4^+-N (**a**), NO_3^--N (**b**), and soil inorganic nitrogen (SIN) (**c**) contents compared to their initial values between soil water content treatments (80% water holding capacity (WHC) vs. 40% WHC).

3.3. Soil R_a, R_{nit}, and R_{min} under Different SSC, FT Temperatures/Cycles, and SWC

The effects of temperature, salinity, moisture, FTCs, and their interactions on R_a were significant ($p < 0.05$) (Table 4), while R_{nit} was significantly affected by temperature and salinity, and R_{min} was significantly affected by temperature ($p < 0.05$). The changing trends of R_a, R_{nit}, and R_{min} were similar between moisture treatments but were obviously different between different FT temperatures and salinity treatments (Figure 6). R_a exhibited greater change during the first 2 days, but the variation differed in different treatments. The R_a decreased on both day 1 and 2 in C (5 °C), peaked (positive values) on day 1, and decreased to the minimum values (negative values) on day 2 in the FT (−5 + 5 °C) and FT (−10 + 5 °C) treatments, and increased on both days and peaked on day 2 in the FT (−15 + 5 °C) treatments. In addition, the R_a during days 5–8 gradually changed from negative values to positive values with the decrease in the freezing temperature. R_{nit} exhibited a similar increasing trend at both day 1 and day 2, except for that in the FT (−15 + 5 °C) treatment, which decreased on day 1 and increased on day 2. However, R_{nit} varied greatly during the later incubation days. R_{nit} decreased under the C (5 °C) treatment, increased under the FT (−10 + 5 °C) treatments, decreased and then increased under the FT (−5 + 5 °C) treatments, and exhibited the opposite trend under FT (−10 + 5 °C) treatments. The differences in R_{nit} between different incubation periods were less marked than the differences in R_a, and the absolute values of R_{nit} were obviously lower in high salinity and lower freezing temperature treatments. R_{min} in the C (5 °C) treatments exhibited a similar changing trend to R_{nit}, while in the FT treatments, R_{min} showed a similar changing trend to R_a.

Table 4. Results of multi-way analysis of variance (ANOVA) on R_a, R_{nit}, and R_{min} under different incubation freezing–thawing temperatures/cycles, salinity, and soil moisture (T: temperature; SSC: soil salt content; SWC: soil water content; FTC: number of freezing–thawing cycles; R_a: net N ammonification rate; R_{nit}: net soil nitrification rate; R_{min}: net soil nitrogen mineralization rate).

Factors	R_a		R_{nit}		R_{min}	
	F	**Sig.**	***F***	**Sig.**	***F***	**Sig.**
T	224.801	0.000 **	4.190	0.006 *	4.299	0.005 *
SSC	5.949	0.000 **	3.792	0.005 *	1.700	0.150
SWC	23.045	0.000 **	0.112	0.738	0.286	0.593
FTC	635.058	0.000 **	1.234	0.297	1.420	0.237
T × SSC	5.275	0.000 **	1.435	0.148	1.468	0.135
T × SWC	5.948	0.001 *	0.052	0.984	0.022	0.996
T × FTC	518.803	0.000 **	0.339	0.961	0.171	0.997
SSC × SWC	2.438	0.047 *	2.005	0.094	1.343	0.254

Table 4. *Cont.*

Factors	R_a		R_{nit}		R_{min}	
	F	Sig.	*F*	Sig.	*F*	Sig.
SSC × FTC	18.891	0.000 **	1.039	0.412	0.685	0.766
SWC × FTC	18.450	0.000 **	1.333	0.264	0.202	0.895
T × SSC × SWC	1.892	0.035 *	0.426	0.953	0.305	0.988
T × SSC × FTC	13.511	0.000 **	0.120	1.000	0.185	1.000
T × SWC × FTC	16.351	0.000 **	0.364	0.952	0.152	0.998
SSC × SWC × FTC	5.683	0.000 **	0.998	0.45	0.552	0.880
T × SSC × SWC × FTC	9.379	0.000 **	0.175	1.000	0.131	1.000

**, $p < 0.01$; *, $p < 0.05$.

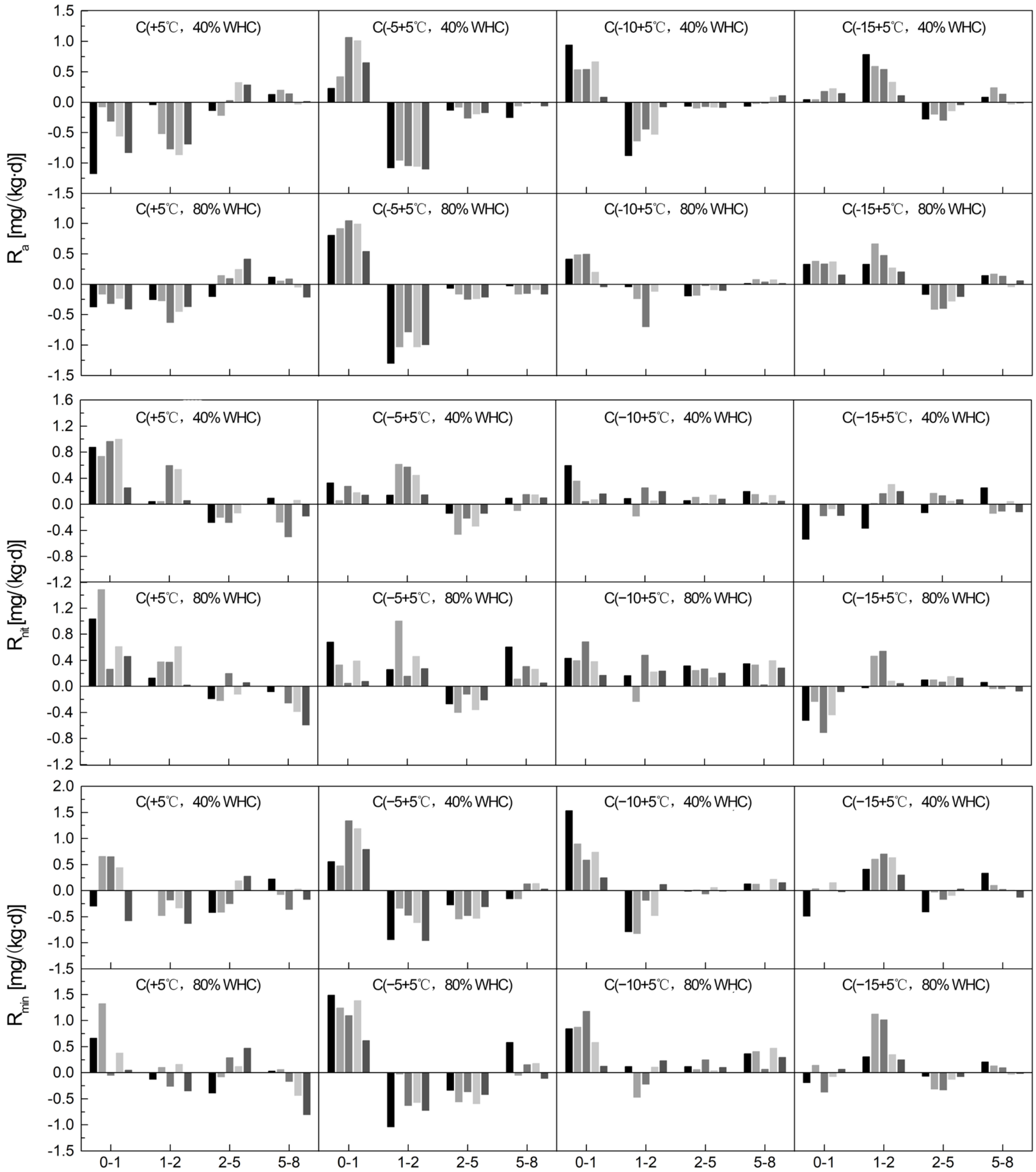


Figure 6. Soil N mineralization and nitrification rates under different soil salt contents (SSC), freezing–thawing (FT) temperatures/cycles, and soil water contents (SWC).

4. Discussion

4.1. Effects of FT Temperatures/Cycles on N Mineralization in Salinized Soil

Soil NH_4^+-N variation and R_a were significantly affected by temperature ($p < 0.05$ and $p < 0.001$, respectively) (Tables 3 and 4), and changed from decreasing trends and negative values in C (5 °C) to increasing trends and positive values in FT treatments during the first 1 or 2 incubation days, respectively. These findings were consistent with those of previous studies. NH_4^+-N was mostly transformed into NO_3^--N in C (5 °C) during the first two incubation days. However, in FT treatments, ice crystal formation in the interstices of the soil and cytosol of microorganisms after immediate freezing could destroy soil aggregates and microbes, enhancing the release of previously unavailable NH_4^+-N from soil inorganic and organic colloids and cell lysis [31,34]. These increases are mostly reported to occur during the first FTC [53] because the residual microbes adapt gradually to the low-temperature conditions with increasing FTCs [54]. Lower freezing temperatures have a greater effect on soil aggregates and microbes, providing more mineralizable organic matter [27,55]. Therefore, the relative NH_4^+-N contents increased with the decreasing of freezing temperature (Figure 4).

The effects of FT temperature on NO_3^--N transformation were significant but nonlinear. The average NO_3^--N content increased by 11.47% after 2 days of incubation in the C (5 °C) treatment, while the increments were 5.06%, 4.20%, and −0.96% in the FT (−5 + 5 °C), FT (−10 + 5 °C), and FT (−15 + 5 °C) treatments, respectively (Figures 2 and A1). This finding indicated that the formation of NO_3^--N was lower than that of C (5 °C) at the beginning of incubation, and decreased with decreasing freezing temperature. The reason was that the formation of NO_3^--N was mostly the combined result of nitrification and denitrification. It has been reported that denitrifying bacteria are more resistant to freezing temperatures than nitrifying bacteria, and the denitrifying function can recover quickly during soil thawing [56,57]. The freezing of soil creates an oxygen-deficient environment more easily because of the formation of ice crystals, which may last longer when freezing temperatures decrease due to the early freezing and slow melting of ice. Therefore, in this study the soil exhibited weaker nitrification and stronger denitrification processes when the freezing temperature decreased, leading to the consumption of NO_3^--N during the FT events with colder freezing temperatures [27,55,58].

The variation trend of NO_3^--N during the following incubation time changed from a decreasing trend in C (5 °C) to a decreasing and then increasing trend in FT (−5 + 5 °C) and a stable increasing trend in FT (−10 + 5 °C) and FT (−15 + 5 °C). However, the increasing trend was weakened in FT (−15 + 5 °C). The reason could be that after 2 days of incubation, the oxygen in the soil was consumed during the nitrification process in the C (5 °C) treatments, and the soil gradually became an anaerobic environment that was favored by denitrifying bacteria [59]. When the soil experienced a mild freezing temperature (−5 °C), although the nitrification bacteria were somewhat inhibited during the freezing process, they recovered quickly and exhibited even stronger activity during the thawing process because more available mineralizable organic matter was released by the destruction of soil aggregates and microbes as discussed above [60]. Therefore, the NO_3^--N still increased during the first 2 days in FT (−5 + 5 °C). During days 2–5, the FT-induced nutrient supply was exhausted, the enhanced function vanished, and denitrification processes regained dominance. During later incubation time (days 6–8), nitrification bacteria gradually adapted to low temperatures, and the NO_3^--N content showed a small increase during this time in FT (−5 + 5 °C). R_{nit} remained high throughout the incubation period when the soil experienced a moderate freezing temperature (−10 °C). This may have occurred because some bacteria that promote nitrification processes at subzero temperatures only display activity at certain temperatures [61]. For example, Juan et al. found that when soil was frozen at −9 °C, the bacterial community could recover rapidly using the labile nutrients produced from an increase in protein depolymerization during soil thawing; in addition, functional change after an FT with a moderate freezing temperature (−9 °C) exactly coincided with the adaptations necessary for the growth of psychrophilic bacteria at low temperatures [35]. Denitrification was significantly related to the bacterial community, which was affected by a severe freezing temperature (−15 °C) more greatly than a moderate freezing temperature, leading to higher denitrification with the freezing temperature of −15 °C [35]. Denitrification consumed NO_3^--N, thereby resulting in a slightly increasing trend of NO_3^--N in FT (−15 + 5 °C) during days 3–8. As a result, the FT process decreased NO_3^--N content compared with C (5 °C) when the freezing temperatures were −5 °C and −15 °C and increased when the freezing temperature was −10 °C (Figure 4).

The variations of SIN and R_{min} in C (5 °C) were similar to those of NO_3^--N and R_{nit}, while they were more consistent with those of NH_4^+-N and R_a in the FT treatments. This finding indicated that R_{min} was more affected by the change of NH_4^+-N during the FT process. Therefore, the SIN contents increased under FT (−10 + 5 °C) and FT (−15 + 5 °C) treatments because their NH_4^+-N contents were significantly enhanced by lower freezing temperatures, as discussed above (Figure 3a).

4.2. Effects of SSC and SWC on Nitrogen Mineralization during the FT Process

The relative NH_4^+-N content increased with the increase in SSC (Figure 3a,d) in the C (5 °C) treatments. This was because increasing salinity completely inhibited nitrification, but did not completely inhibit ammonification [62], and increased pore water salinity due to salt addition can desorb exchangeable ammonium [63,64]. In addition, dissimilatory nitrate reduction to ammonium (DNRS) increased with rising salinity [65], which may have also increased the NH_4^+-N content at high SSC. Under the FT treatments, NH_4^+-N increased with the decrease in freezing temperature, and the increment was higher in low-SSC treatments (Figure 3a,d and Figure 4). The relationship between the relative NH_4^+-N content and SSC changed from an increasing trend to a decreasing trend (Figure 3), and the variation of relative NH_4^+-N content induced by freezing temperature decreased with enhanced salt addition (Figure 4a). These findings indicated that enhanced salinity inhibited the positive effect of freezing temperature on NH_4^+-N formation. The reason was that enhanced SSC decreased the freezing temperature of soil water, decreasing the formation of ice crystals and resulting in more remaining liquid water [41–43]. These functions reduced the destruction of soil aggregates and the death of microbes [29,30,34], decreased the release of nutrients such as active organic carbon and inorganic nitrogen [66], which thereby weakened their stimulation of enzyme activity [29], and finally decreased the NH_4^+-N formation. Another reason for the inhibition was that the liquid water increment induced by the addition of salt to frozen soil allowed more nitrifying bacteria to survive, and the decreased ice crystal formation reduced the obstruction of soil voids, thus reducing the inhibition of freezing temperature on NO_3^--N formation and the consumption of NO_3^--N by denitrification [67,68]. As a result, the relative NO_3^--N content gradually changed from decreasing with SSC to increasing with increased SSC (Figure 4b,e). The stronger nitrification in the high-SSC treatments consumed more NH_4^+-N, and further reduced the NH_4^+-N content.

The relative NH_4^+-N content of different salinity treatments increased linearly with the decrease in freezing temperature (Figure 7). The fitting formulas are depicted in Table 5. The slopes of these fitting lines were good indicators to evaluate the combined effect of SSC and freezing temperature on the relative NH_4^+-N content. Higher absolute values of the slope indicated a greater increase in NH_4^+-N with decreased freezing temperatures. As shown in Figure 7c, the absolute value of the slope decreased with increasing SSC, further demonstrating that enhanced salinity inhibited the effect of freezing temperature on NH_4^+-N formation, as discussed above. Moreover, the increase in SWC significantly decreased the absolute values of the slope, especially in the high-salinity treatments, indicating that the increase in SWC could weaken the NH_4^+-N formation induced by the decrease in freezing temperature, and this function increased with the increase in salinity (Figure 7c). These findings corresponded to the phenomenon observed in Figure 5, in which the increase in SWC enhanced the production of NH_4^+-N in the unfrozen and low-SSC FT treatments, and reduced the production of NH_4^+-N in the high-SSC combined with low-freezing temperature treatments (Figure 5a). The reason could be that the increase in soil moisture from 40% WHC (the maximum water holding capacity) to 80% WHC could relieve the water-limited activity and substrate transport of microbes [1,69] and enhance NH_4^+-N and NO_3^--N contents in low-SSC treatments of C (5 °C). The increase in soil moisture also reduced the salt concentration of soil water but created a more severe anaerobic environment, which enhanced the activity of urease and denitrifying bacteria [4]. As a result, the NH_4^+-N content increased, while the NO_3^--N contents remained unchanged or decreased in high-SSC treatments of C (5 °C) (Figure 5a,b). In the FT treatments, enhanced SWC could increase soil temperature by increasing the heat capacity of soil during the freezing period [70]. Higher initial SWC and SSC induced higher liquid water content in frozen soil [39]. Therefore, the harm caused by FT to soil aggregates and microbes was weakened, and the FT-induced NH_4^+-N was greatly reduced in treatments with high SSC and SWC at low freezing temperatures. In addition, soils with a higher SWC had higher freezing points and would be prone to freezing but resistant to thawing, thereby increasing the frost duration [70,71]. Longer frost duration provided more time for facultative bacteria to switch from aerobic to anaerobic activities, which was also enhanced by the lower salinity induced by higher liquid water content [33,72]. Consequently, the relative NO_3^--N contents were mostly reduced in treatments with high SSC and low freezing temperatures when SWC increased (Figure 5b).

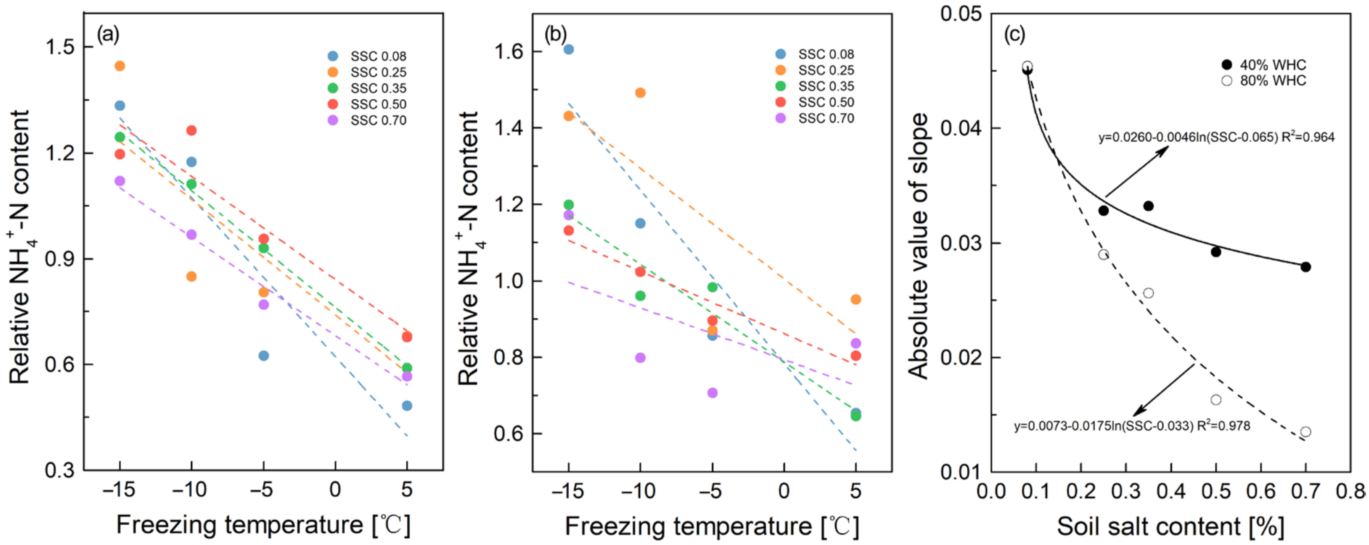


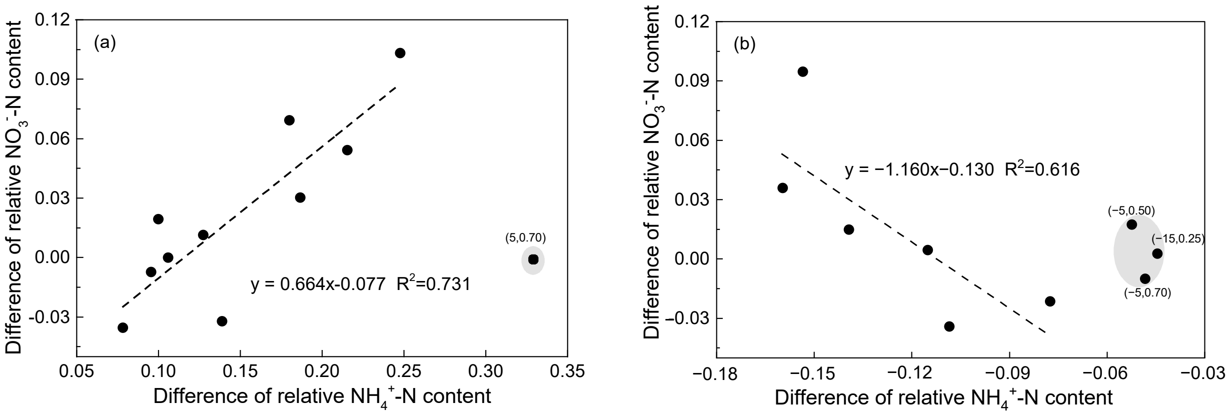
Figure 7. Relationship between relative NH_4^+-N content and freezing temperature under soil water content treatments ((**a**) 40% water holding capacity (WHC) vs. (**b**) 80% WHC), and (**c**) the relationship between soil salt content (SSC) and the absolute values of their fitting line slopes.

Table 5. Fitting lines of relative NH_4^+-N content and freezing temperature (SWC: soil water content; WHC: water holding capacity; SSC: soil salt content).

SSC	SWC = 40% WHC			SWC = 80% WHC		
	Equation: Y = Relative content	R	Sig	Equation: Y = Relative Content	R	Sig
0.08	Y = −0.0451T + 0.6226	0.8659	0.035 *	Y = −0.0454T + 0.7826	0.8821	0.030 *
0.25	Y = −0.0328T + 0.7401	0.6724	0.090	Y = −0.029T + 1.0054	0.5937	0.115
0.35	Y = −0.0332T + 0.7615	0.9975	0.001 **	Y = −0.0256T + 0.7874	0.9206	0.020 *
0.50	Y = −0.0292T + 0.8411	0.8813	0.031 *	Y = −0.0163T + 0.8616	0.9443	0.014 *
0.70	Y = −0.0279T + 0.6816	0.9783	0.005 *	Y = −0.0135T + 0.7936	0.3240	0.215

**, $p < 0.01$; *, $p < 0.05$.

The relationships between the differences in relative NH_4^+-N and NO_3^--N content caused by increased SWC in treatments with low SSC (L-SSC) × high freezing temperature (H-FTem) and high SSC (H-SSC) × low freezing temperature (L-FTem) are shown in Figure 8a,b, respectively. The difference in the relative NH_4^+-N content was positively correlated with that of NO_3^--N, indicating that the increases in NH_4^+-N and NO_3^--N in L-SSC × H-FTem treatments induced by SWC addition mostly occurred during the thawing period, as discussed above. The negative relationship between the changes of NH_4^+-N and NO_3^--N in H-SSC×L-FTem may indicate that the dissimilatory nitrate reduction to ammonium (DNRA) occurred, as it has been reported to increase with the increase in SWC [73] and salinity [65].



(**a**) (**b**)

Figure 8. Relationship between the relative NH_4^+-N and NO_3^--N content differences caused by increased soil water content (SWC) (40–80% water holding capacity (WHC)) in treatments with low soil salt content (SSC) and high freezing temperature (**a**) and in treatments with high SSC and low freezing temperature (**b**).

The inconsistent effects of FT, SSC, and SWC on NH_4^+-N and NO_3^--N led to different variations of SIN. For example, NH_4^+-N and NO_3^--N contents were increased and decreased with SSC in C (5 °C), respectively, resulting in an increasing and then decreasing trend of SIN in C (5 °C). The SIN content was higher in FT (−15 + 5 °C) than in C (5 °C) when the SWC was 40% WHC, while the SIN content was lower in high-SSC treatments when SWC increased to 80% WHC because the combined effect of enhanced SWC and SSC reduced both NH_4^+-N and NO_3^--N contents as shown in Figure 5 and discussed above.

5. Conclusions

Soil water content, temperature, and salinity have complex interactions on soil inorganic nitrogen transformation during FT processes, and these interactions have inconsistent effects on NH_4^+-N, NO_3^--N, and SIN. The FT processes increased relative NH_4^+-N content by 13%, 39%, and 77% with the decreasing of freezing temperature from −5 °C to −15 °C compared with C (5 °C) treatments, respectively. FT (−5 + 5 °C) and FT (−15 + 5 °C) treatments decreased the relative NO_3^--N contents by 4% and 6% compared with C (5 °C) treatments, respectively. Under FT treatments, the increment of relative NH_4^+-N content was higher in low-SSC treatments and lower in high-SSC treatments, indicating that enhanced salinity inhibited the positive effects of freezing temperature on NH_4^+-N formation. The relationship between relative NO_3^--N content and SSC gradually changed from a decrease in the C (5 °C) treatments to an increase in the FT (−15 + 5 °C) treatments, indicating that enhanced salinity inhibited the negative effects of freezing temperature on NO_3^--N formation. In addition, the increase in SWC could weaken the trend of NH_4^+-N formation induced by the decrease in freezing temperature, and this function increased with the increase in salinity. The variations of SIN and R_{min} in the C (5 °C) treatments were similar to those of NO_3^--N and R_{nit}, while they were more consistent with those of NH_4^+-N and R_a in the FT treatments. These findings are valuable to guide the management of fertilizer and water in seasonal FT saline farmland. Further studies combining laboratory and field experiments that include more multi-gradient controlling factors are needed to improve the understanding of nitrogen transformation in seasonally freezing salinized farmland ecosystems.

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Data Availability Statement: The datasets generated during and/or analysed during the current study are not publicly available due to the confidential nature of the data but are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

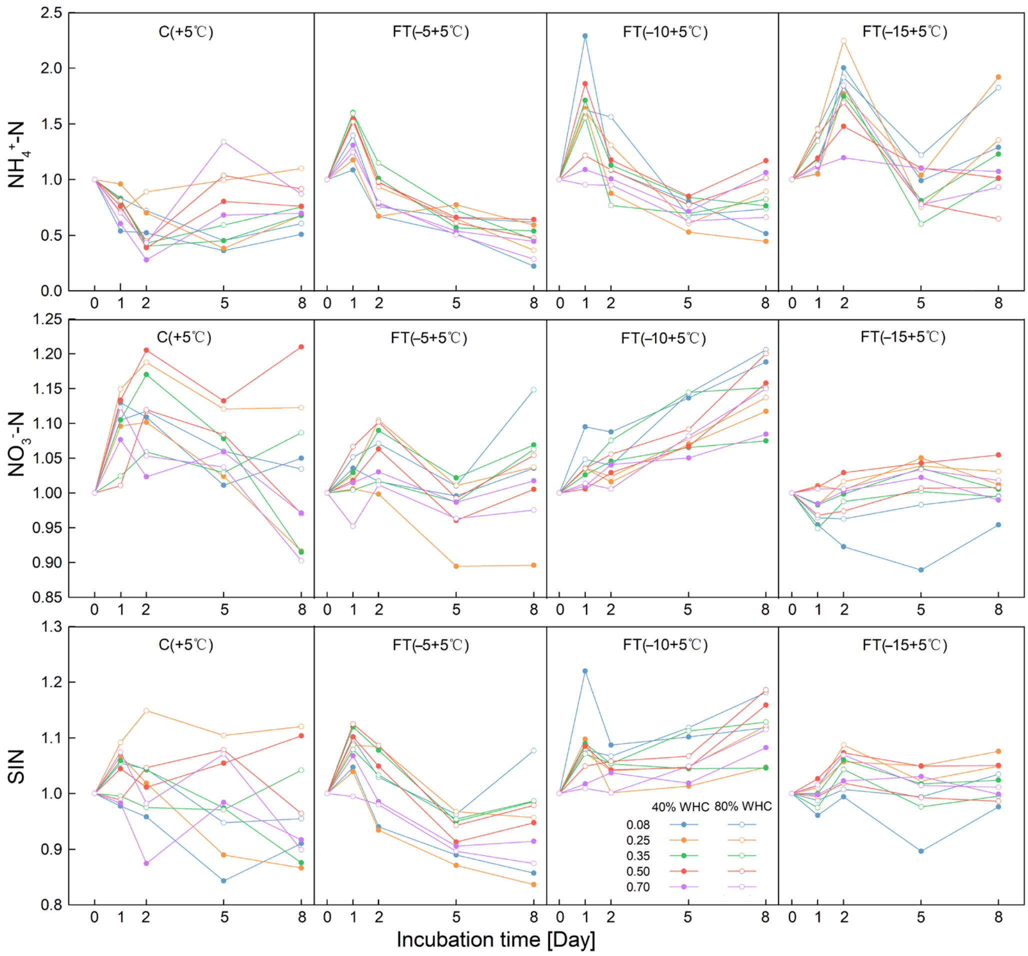

Figure A1. Ratio of soil NH_4^+-N, NO_3^--N, and soil inorganic nitrogen (SIN) contents to their initial values during the incubation period under different salinity, freezing–thawing temperatures/cycles, and soil moisture content.

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