Pol. J. Environ. Stud. Vol. 33, No. 5 (2024), 1-12
DOI: *10.15244/pjoes/185694* ONLINE PUBLICATION DATE:

Original Research

Nitrification and Urease Inhibitors Reduce the Stimulated Nitrous Oxide Emissions by the Freeze-Thaw Cycles

Youlong Xu[1†], Fei Xia[2,3†], Yunyin Xue[1†], Junqiang Wang[1*], Yuanyuan Zhao[1], Yanfang Zhou[4], Linling Ran[1], Haoyang Wu[1] , Ziteng Xie[1], Jianyao Li[1],

[1] Key Laboratory of Southwest China Wildlife Resource Conservation (Ministry of Education). China West Normal University, Nanchong, 637009, P.R. China
[2] State Key Laboratory of Highland Barley and Yak Germplasm Resources and Genetic Improvement, Lhasa, 850000, P.R. China
[3] Institute of Pratacultural Science, Tibet Academy of Agriculture and Animal Husbandry Science, Lhasa, 850000, P.R. China
[4] Gansu Provincial Institute of Agricultural Engineering and Technology, Wuwei,733006, P.R. China

Received: 27 October 2023
Accepted: 05 March 2024

Abstract

Most studies have demonstrated that nitrification and urease inhibitors can reduce soil nitrous oxide (N_2O) emissions from nitrogen-fertilized farmland. However, few studies have examined the potential impacts of these inhibitors on semi-arid agricultural farmland in the presence of freeze-thaw (FT) cycles. The purpose of this study was to assess the efficacy of applying the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) and the urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT) to soil nitrogen transformation and studying N_2O emissions through simulated indoor FT incubation to offer theoretical and technological guidance for mitigating nitrogen loss in semi-arid farmland. The results showed that urea with DMPP under freeze-thaw conditions significantly increased the inorganic nitrogen content of the soil, kept the ammonium nitrogen content of the soil at a high level, suppressed the net nitrification rate of the soil, and reduced the cumulative emission of nitrous oxide (N_2O) in the soil by nearly 87.6% compared to CK. Urea incubation with NBPT under freeze-thaw conditions also significantly reduced fluxes and cumulative N_2O emissions. Due to the dual inhibition of soil nitrification rate by DMPP/NBPT and the FT cycle, the addition of DMPP/NBPT during soil FT could alleviate soil N_2O emission caused by the effect of the FT cycle after urea addition and reduce soil nitrogen loss. The results indicate that the application of DMPP/NBPT can effectively alleviate the irrigated silt soil N_2O emission during the FT period.

Keywords: Freeze-thaw, Nitrogen, DMPP, NBPT, N_2O emission

[†] These authors contributed equally to this work
* e-mail: wangjunq0303@163.com

Introduction

Urea is often the N fertilizer choice in conventional irrigated systems in Northwest China because of its high N concentration and favorable cost [1]. When applied to soil, commonly as granules, only 40% of the applied N is recovered by crops globally [2]. Most of the unrecovered fertilizer N by plants may be released as N_2O through several biochemical processes, but there remains a poor understanding of their regulation and variation among soil types [3], causing a series of adverse consequences, including air pollution and climate change. Therefore, strategies to stabilize urea and improve N availability in the plant-soil system are needed to meet corn N demand and mitigate environmental pollution. To reduce N losses and increase fertilizer N use efficiency, urease inhibitors [4] and nitrification inhibitors [5] have been introduced to agricultural soils.

Urease inhibitors, such as N-(n-butyl) thiophosphoric triamide (NBPT), have been developed to minimize N loss by inhibiting urease enzyme activity, delaying urea hydrolysis, and preventing the elevated pH that drives NH_3 loss [4]. Nitrification inhibitors, such as 3,4 dimethylpyrazole phosphate (DMPP), can slow the conversion of NH_4^+-N to NO_3^--N by decreasing the enzymatic activity of ammonium-oxidizing bacteria, leading to a reduction in nitrification and the emission of N_2O from soils [6]. In a meta-analysis using data from 12 countries, Silva *et al.* (2017) reported that the application of urea with NBPT decreased average cumulative NH_3 losses by 52% across a range of soil pH values [7], soil textures, organic carbon contents, N rates, and inhibitor concentrations because it reduced the activity of the urease enzyme and therefore slowed the hydrolysis of urea, leading to a decrease in the volatilization of ammonia (NH_3) from soils. In a global meta-analysis, Wu *et al.* (2021) found that the application of urea with a nitrification inhibitor (e.g., dicyandiamide, DCD) decreased N loss by N_2O emissions [8], but furthermore, when urea was applied with both urease and nitrification inhibitors, both NH_3 volatilization and N_2O emissions were reduced, thus avoiding pollution swapping [9]. Conflicting results exist regarding the impact of NBPT on N_2O emissions and N loss, with some studies showing no effect [10] and others reporting positive effects [11]. Some results indicate that N_2O emissions were partly derived from below-ground sources of N not affected by DMPP [12]. Thus far, little information is available on the potential of the combined application of DMPP and NBPT to reduce greenhouse gas (GHG) emissions.

Freeze-thaw (FT) cycle events mainly occur at high latitudes, high altitudes, and in some temperate regions [13]. Approximately 55% of the total land area in the Northern Hemisphere experiences seasonal soil freezing [14]. Many research articles have reported that the FT process can alter soil nitrogen pools and stimulate nitrogen turnovers, such as mineralization, nitrification, and the emission of nitric gas in croplands [15, 16]. Freeze-thaw cycles can lead to a depletion of O_2 in the soil, stimulating N_2O production via denitrification [17]. It has been demonstrated that N_2O emissions associated with freeze-thaw cycles can account for 20-90% of annual emissions [18], and neglecting freeze-thaw emissions would underestimate agricultural N_2O emissions by 17-28% [19]. Some studies conducted in natural as well as agricultural systems have addressed how FT cycles can lead to an increase in N_2O emissions [20]. Notably, only 25% of the included data covered an entire year, including both fallow and growing seasons, in the meta-analysis conducted by Ruser and Schulz (2015) [21]. The effects of nitrification and urease inhibitors summarized in these studies were mostly limited to measurements made only during the crop-growing season.

To our knowledge, some studies investigating the effect of urease and nitrification inhibitors on N_2O in rainfed maize soil have been reported [22]. However, studies on the effect of FT combined with urease and nitrification inhibitor application on nitrogen mineralization, nitrification, and denitrification are relatively insufficient, and therefore, it is still unclear how repeated FT cycles will influence these responses. Thus, we conducted an incubation using typical arable soil and a combined method inhibiting N_2O production (NBPT and DMPP) to distinguish the relative contributions of FT cycles to soil N_2O emissions in the context of inorganic N (urea) amendments. We hypothesized that (1) FT cycles can alter nitrogen transformation processes in irrigated silt soils, and (2) DMPP and NBPT applications provide the same opportunity to reduce soil N_2O emissions from agricultural ecosystems during FT periods.

Materials and Methods

Site Description and Soil Collection

The experimental soil samples were collected from Minqin County in Gansu Province, China (103°07′00.16″E, 38°37′10″N) (Fig. 1), located within the lower reach of the Shiyang River basin in the Hexi Corridor and bound by the Tenggeli and Badanjaran Deserts [23]. The area is characterized by a typical arid continental climate with a mean annual air temperature of 7.8 °C, a mean annual precipitation of 113.2 mm, most of which falls between July and September, and an annual average evaporation of 2,646 mm.

The soil at the research site is irrigated desert soil according to the Chinese Soil Classification System and is similar to Anthropic Camborthids according to Soil Taxonomy [24]. Other relevant environmental conditions and agricultural production in the study area are described by Feng et al. (2011) [23]. Soil samples were collected from conservation tillage arable lands set up in 2014 (Fig. 1) with flat planting of spring maize (*Zea mays* L.). At the start of the experiment, the soil had a pH of 8.63, 0.29 ms cm^{-1} specific conductance, 9.80 g kg^{-1} organic matter, 4.84 g kg^{-1} total N, 0.40 mg kg^{-1} available phosphorus (P), and 144.14 mg kg^{-1} available potassium (K).

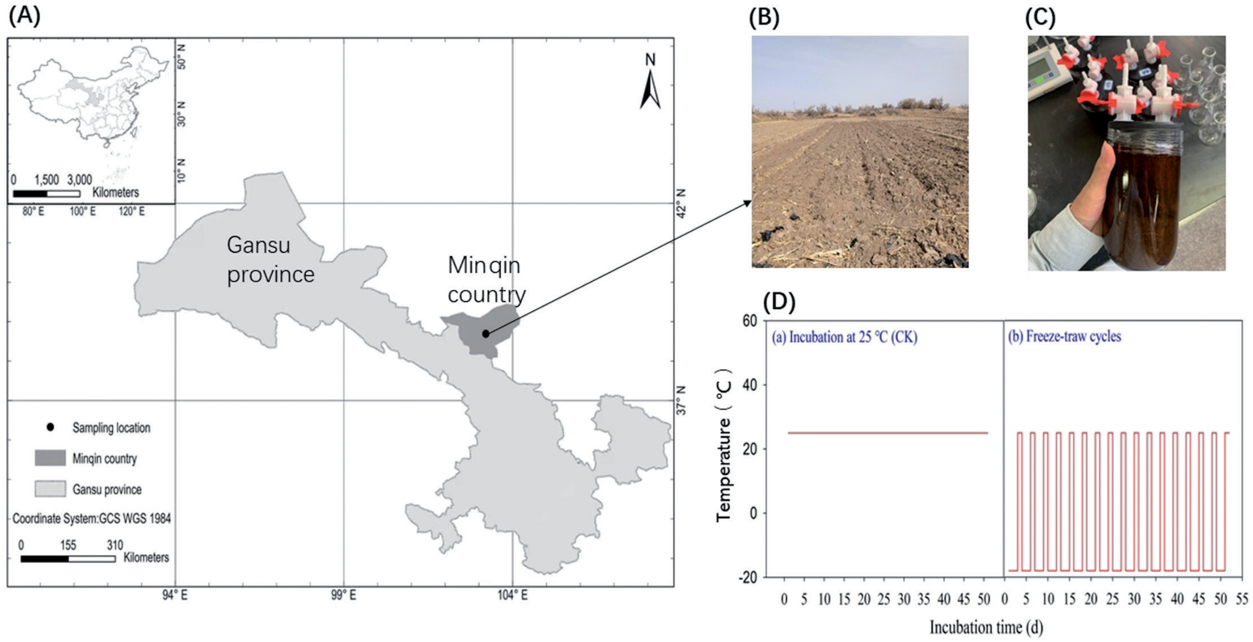

Fig. 1. Location of sampling sites (A-B), experimental pre-culture (C), and temperature treatments (D).

Soil samples were taken from the plow layer (0-20 cm) using a 10 cm diameter auger at five randomly selected points in April 2021 and mixed to create a composite sample per plot. The visible roots and rocks were removed in this step.

Soil Incubation Experiment and Sampling

The collected soil samples were dried at 4°C to maintain soil biological activity, crushed, and passed through a 5-mm nylon fiber soil sample sieve before incubation. The incubation experiments were conducted in 500 mL glass incubation flasks (Fig. 1), and before incubation, soil samples equivalent to 250 g portions of oven-dry soil were adjusted to approximately 15% of the maximum water-holding capacity (WHC) and preincubated at 25°C for 7 days to restore soil microbial activity. Soils were mixed with urea (N 46.0%), urea +NBPT, and urea + DMPP (urea at a rate of 300 mg N kg^{-1} soil and NBPT and DMPP at a rate of 2% of urea-N) and incubated under different FT cycles (freezing at -18°C for 48 h and thawing at 25°C for 24 h, referred to as one FT cycle, Fig. 1d). Sterile water was added to the soil by weighing it to determine 60% of WHC during incubation periods. There were six treatments in total (FUR: urea addition with FT cycles; RDP: urea + DMPP addition with a constant temperature of 25°C; FDP: urea + DMPP addition with FT cycles; RNP: urea + NBPT addition with a constant temperature of 25°C; FNP: urea + NBPT addition with FT cycles; CK: addition with urea and continuous incubation at 25°C). Three replicates were prepared for each treatment.

Destructive sampling was performed after one FT cycle (3 days), three FT cycles (9 days), twelve FT cycles (36 days), and seventeen FT cycles (51 days). To measure the flux of N_2O, air exchange between the inside and outside of the incubation flasks was stopped 2 h before the end of the freeze-thaw cycle, and the flask gas exchange valve was closed. When the incubation was finished, the gas in the incubation bottles was extracted with a 50 mL syringe and transferred to a 100 mL aluminum foil gas collection bag to send samples for measurement. After destructive soil sampling, part of the soil samples was stored in a 4°C refrigerator for the determination of soil enzyme activity and microbial biomass carbon and nitrogen analyses, and part was air-dried and used for the determination of soil physical and chemical properties.

N_2O Flux Measurements

N_2O concentrations were measured using a gas chromatograph (Varian CP-3800, Palo Alto, CA, USA) equipped with thermal conductivity (TCD), flame-ionization (FID), and electron capture (ECD) detectors, which assessed the carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) levels. The GHG concentration was calculated using the following equation: $F = \beta \times (c/t) \times v \times 273/(W \times (273 + T))$, where F denotes N_2O (μg N_2O-N kg^{-1} h^{-1}); β denotes N_2O (1.962 g/L) density in a standard state; c/t denotes the N_2O (ppb h^{-1}) accumulation rate; v denotes the volume of the gas in the flask; W denotes the dry weight (kg) of soil in the flask; and T denotes the temperature inside the chamber during sampling [25].

Soil Properties, Enzyme Activities, and Soil Microbial Biomass

Soil organic carbon (SOC) was measured using the H_2SO_4-K_2CrO_7 oxidation method, while soil total nitrogen (TN) was detected using an automatic azotometer (Kjeltec 8400, FOSS, Denmark) according to the Kjeldahl method. Soil ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were determined using a continuous flow analyzer (AA3, SEAL Analytical, Germany) with 1 mol/L KCl extracts. Soil-dissolved organic C (DOC) was extracted with deionized water in a 1:10 soil–water ratio, filtered through a 0.45-µm filter, and measured by a TOC analyzer (vario TOC Cube, Elementar Analysensysteme GmbH, Hanau, Germany). Soil pH was measured in water (1:5 w/v) using a pH electrode.

The soil enzymes, including leucine aminopeptidase (LA) and β-1,4-N-acetylglucosaminidase (NAG), were measured following the method of [26]. The activity of urease was measured using the phenol-sodium hypochlorite colorimetric method. All absorbance values for the measurement of soil enzyme activities were read using a multimode microplate reader (Scientific Fluoroskan Ascent FL, Thermo). Soil microbes were extracted from the soil using the chloroform fumigation and extraction method [27], and then the microbial biomass carbon (MBC) and nitrogen (MBN) were measured using an elemental analyzer (Elementar Vario EL III CHNOS, Germany).

Net N Mineralization Rate and Net Nitrification Rate of Soils

The net nitrogen mineralization rate and net nitrification rate of soil were calculated by the following equations [28].

$$A_{amm}(mg\ kg^{-1}) = c[NH_4^+ - \mathrm{N}]_{i+1} - c[NH_4^+ - \mathrm{N}]_i$$

$$A_{nit}(mg\ kg^{-1}) = c[NO_3^- - \mathrm{N}]_{i+1} - c[NO_3^- - \mathrm{N}]_i$$

$$R_{min}(mg\ kg^{-1}\ d^{-1}) = \frac{A_{amm} + A_{nit}}{t_{i+1} - t_i}$$

$$R_{nit}(mg\ kg^{-1}\ d^{-1}) = \frac{Anit}{t_{i+1} - t_i}$$

Where t_i denotes the time before incubation (d), t_{i+1} denotes the time after incubation (d), and $c[NH_4^+\text{-N}]_i$ and $c[NH_4^+\text{-N}]_{i+1}$ denotes the content of NH_4^+-N (mg kg^{-1}) before and after incubation, respectively, $c[NO_3^-\text{-N}]_i$ and $c[NO_3^-\text{-N}]_{i+1}$ denotes the content of NO_3^--N before and after incubation, respectively (mg kg^{-1}), A_{amm} and A_{nit} denote the cumulative NH_4^+-N and NO_3^--N before and after

Table 1. Soil pH, total dissolved nitrogen (TDN), total nitrogen (TN), soil organic carbon (TOC), and microbial biomass nitrogen (MBN) relating to different treatments during four incubation times. Means ± SE (n=12) followed by different letters are significantly different at $p < 0.05$.

Treatments	Incubation time (d)	pH	TDN (mg kg^{-1})	TN (g kg^{-1})	MBN (mg kg^{-1})	TOC (g kg^{-1})
CK	3	8.97±0.03 a	248.4±8.51 d	0.64±0.05 a	124.03±10.78 a	5.36±0.20 a
	9	8.71±0.12 b	407.64±38.58 bc	0.66±0.11 a	135.59 ±20.17a	5.51±0.44 a
	36	7.87±0.08 d	452.29±70.68 a	0.57±0.05 a	105.46 ±34.02 a	5.28±0.33 a
	51	8.18±0.01 c	330.06±23.15 c	0.49±0.06 a	41.13 ±9.79 b	5.30±0.40 a
RDP	3	9.08±0.01 a	160.12±11.02 a	0.61±0.02 a	17.11±3.34 c	5.59±0.60 a
	9	9.03±0.06 a	147.19±4.39 ab	0.55±0.00 b	43.61 ±2.34 a	5.61±0.30 a
	36	8.12±0.24 c	156.64±9.43 ab	0.56±0.01 b	36.69 ±4.56 b	5.89±0.61 a
	51	8.52±0.04 b	138.81±13.11 b	0.58±0.04 bc	34.83 ±1.79 b	5.37±0.66 a
RNP	3	8.91±0.01 a	328.50±7.51 b	0.71±0.03 a	45.24±3.37 b	5.37±0.39 a
	9	8.84±0.02 a	314.28±19.23 b	0.59±0.09 c	91.76±11.40 a	5.41±0.36 a
	36	7.94±0.07 b	399.52±21.80 a	0.61±0.01 ab	89.39±10.77 a	5.49±0.31 a
	51	8.02±0.18 b	326.92±16.5 b	0.59±0.06 c	105.75±8.95 a	5.55±0.15 a
FUR	3	8.91±0.07 a	245.06±2.32 b	0.59±0.07 a	128.36±5.81 a	5.35±0.20 ab
	9	8.79±0.07 b	254.51±61.11 b	0.58±0.01 a	111.45±17.89 a	5.55±0.55 ab
	36	7.98±0.07 d	449.91±30.46 a	0.53±0.11 a	22.85±1.93 b	5.95±0.52 ab
	51	8.14±0.01 c	495.53±94.06 a	0.49±0.02 a	17.85±0.72 b	5.02±0.26 b
FDP	3	9.02±0.03 a	163.46±5.32 ab	0.56±0.03 a	22.11±2.40 c	5.54±0.60 a
	9	9.02±0.02 a	124.16±3.23 b	0.51±0.08 a	38.41±5.89 b	5.38±0.40 a
	36	8.36±0.02 c	196.11±45.49 a	0.52±0.03 a	43.12±2.26 ab	5.52±0.42 a
	51	8.57±0.03 b	176.04±32.51 ab	0.47±0.02 a	47.97±2.08 a	5.01±0.71 a
FNP	3	8.93±0.03 a	326.17±9.70 a	0.70±0.03 b	28.24 ±2.04 b	5.37±0.39 bc
	9	8.86±0.02 b	249.89±14.76 b	0.75±0.02 a	21.06 ±6.30 bc	5.22±0.48 c
	36	8.13±0.06 d	364.44±61.29 a	0.69±0.01 b	19.25 ±3.15 c	6.21±0.52 a
	51	8.42±0.03 c	385.34±31.32 a	0.61±0.04 c	37.18±4.82 a	6.12±0.16 ab
Effect of Treatments		<0.001	<0.001	<0.001	<0.001	0.295
Effect of Incubation time		<0.001	<0.001	<0.001	0.012	
Effect of Treatments × Incubation time <0.001 <0.001		<0.001	0.116	<0.001	0.289	

The significant effect of treatments, incubation time and their interaction are tested by Scheirer Ray Hare test.

incubation, respectively (mg kg^{-1}), and R_{min} and R_{nit} denote the net N mineralization rate and net N nitrification rate (mg kg^{-1} d^{-1}), respectively.

Data Analysis

Experimental data were collated and summarized using Microsoft Excel 2019, and all statistical analyses were performed using IBM SPSS Statistics 21.0 software for the significance of differences, followed primarily by plotting using the R package ggplot2. The random forest algorithm was applied to estimate the relative importance of soil abiotic and biotic control factors on soil nitrous oxide emission fluxes for all incubation times, and we ran the random forest algorithm 100 times to assess the increase in mean square error (lnMSE) and thus the importance of each driver for soil nitrous oxide emission fluxes. The random forest algorithm was executed using the R package *randomForest*, where we set *ntree* and node size to 500 and 5, respectively. To check the *p* value of the importance of each driver, *rfPermute* was used in the random forest algorithm. Finally, we performed a principal component (PCA) analysis using the R packages *factoextra* and *FactoMineR* to reduce the number of variables to simplify the analysis and facilitate interpretation.

Results

Soil's Main Nitrogen-Related Indexes

Compared with 3-day incubation, pH was significantly lower in 51-d incubation for all treatments ($P<0.05$; Table 1), and MBN was significantly lower in the FUR and CK treatments ($P<0.05$; Table 1). The TN content for CK, FUR, and FDP was maintained at 0.47-0.64 g kg^{-1} throughout the incubation period (Table 1), but FDP and FNP treatments had significant effects on TN content. Treatments, incubation time, and their interaction had significant effects on pH, TDN, and MBN concentrations ($P<0.001$, Table 1), whereas there was no effect on TOC content. Urea

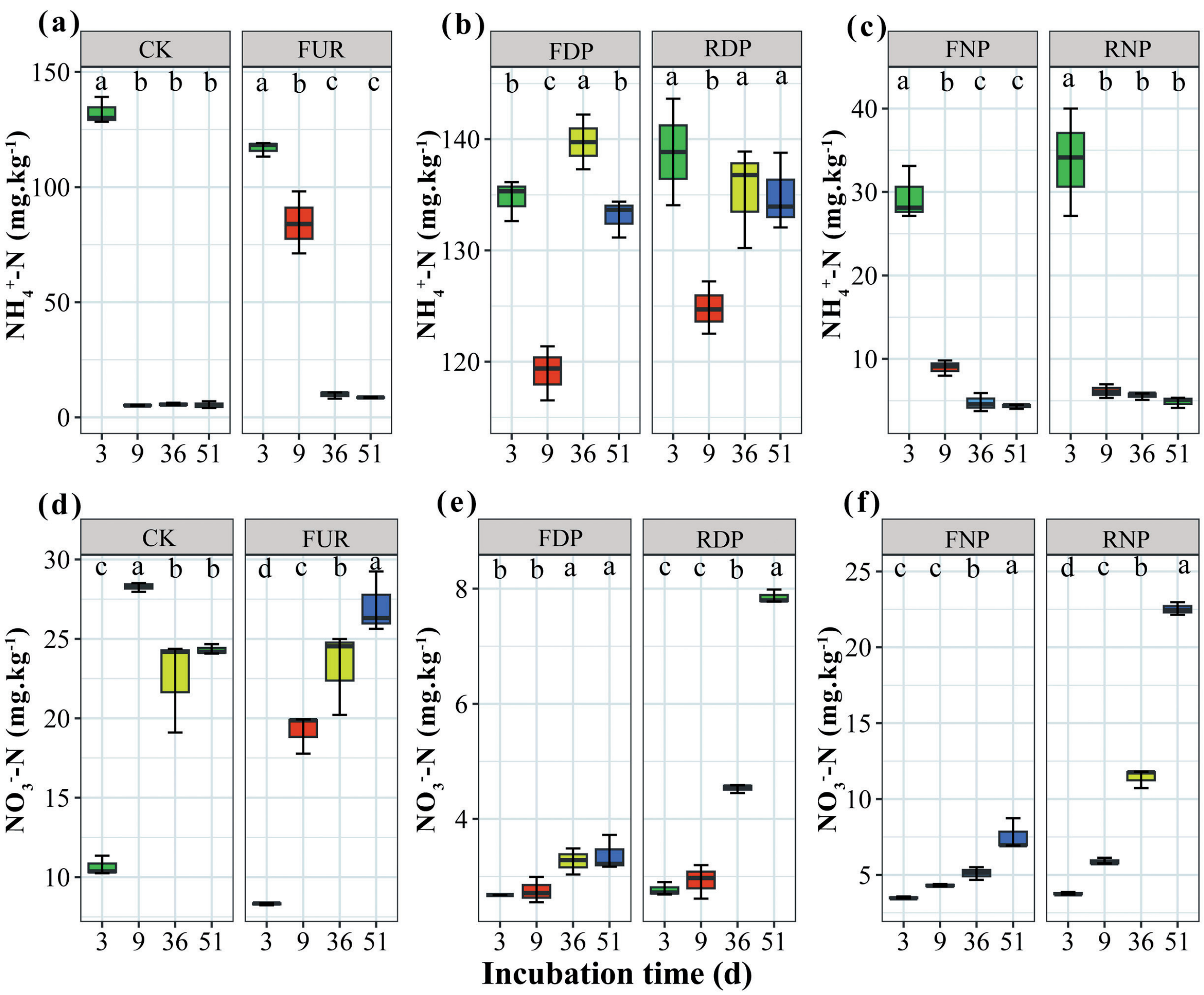

Fig. 2. Changes in soil ammonium and nitrate nitrogen contents under different treatments during the incubation period (mean ± SE, n = 3). Different lowercase letters indicate significant differences (p<0.05) between different incubation times under the same incubation treatment.

addition and urea addition+NBPT significantly increased soil NH_4^+-N concentration at 3 days after incubation; in contrast, the lowest soil NO_3^--N concentration was observed at 3 days of incubation (P<0.001, Fig. 2).

Soil Nitrogen Mineralization and Nitrification Characteristics

The net N mineralization rate of soil reached its peak at 3 days of incubation (Fig. 3a). Compared with CK, the net N mineralization rates were significantly lower in the RNP, FNP, and FUR treatments (P<0.05; Fig. 3). On 9 days of incubation, the net N mineralization rates were all negative, indicating the occurrence of N fixation (Fig. 3a.) on 3 days and 9 days of incubation, the net nitrification rate for all treatments was significantly lower than that of CK (P<0.05; Fig. 3c). Throughout the incubation, the FDP and FNP net nitrification rates were lower than those of FUR (Fig. 3c).

The cumulative nitrogen mineralization for RDP and FDP was significantly higher than that of the CK treatment, and the cumulative nitrogen mineralization of RNP, FUR, and FNP was not significantly different from that of CK (P<0.05; Fig. 3b), but the cumulative nitrification for RDP and FDP was significantly lower than that of the CK treatment, and the cumulative nitrification of FUR was significantly higher than that of the CK treatment (P<0.05; Fig. 3d).

N_2O Emission Fluxes and Accumulation

Throughout the incubation, N_2O emissions in the RDP, RNP, FDP, and FNP were relatively low compared to CK (Fig. 4a). The soil cumulative N_2O emissions in the FUR treatment were significantly higher than other treatments (P<0.05) and reached 10.63 ug kg^{-1} during the incubation period (Fig. 5b). Random forest regression analysis explained 56% and 38% of the variance in the N_2O emission fluxes during the continuous 25°C incubation and freeze-thaw incubation (Fig. 5), respectively. The analysis showed that NH_4^+-N, LA, and urea activity (Urease) were the most important factors that controlled the soil N_2O emissions during the continuous 25°C incubation (Fig. 5a). During the freeze-thaw incubation, NO_3^--N, MBN, NH_4^+-N, and MBC were the most important factors that controlled the soil N_2O emissions (Fig. 5b).

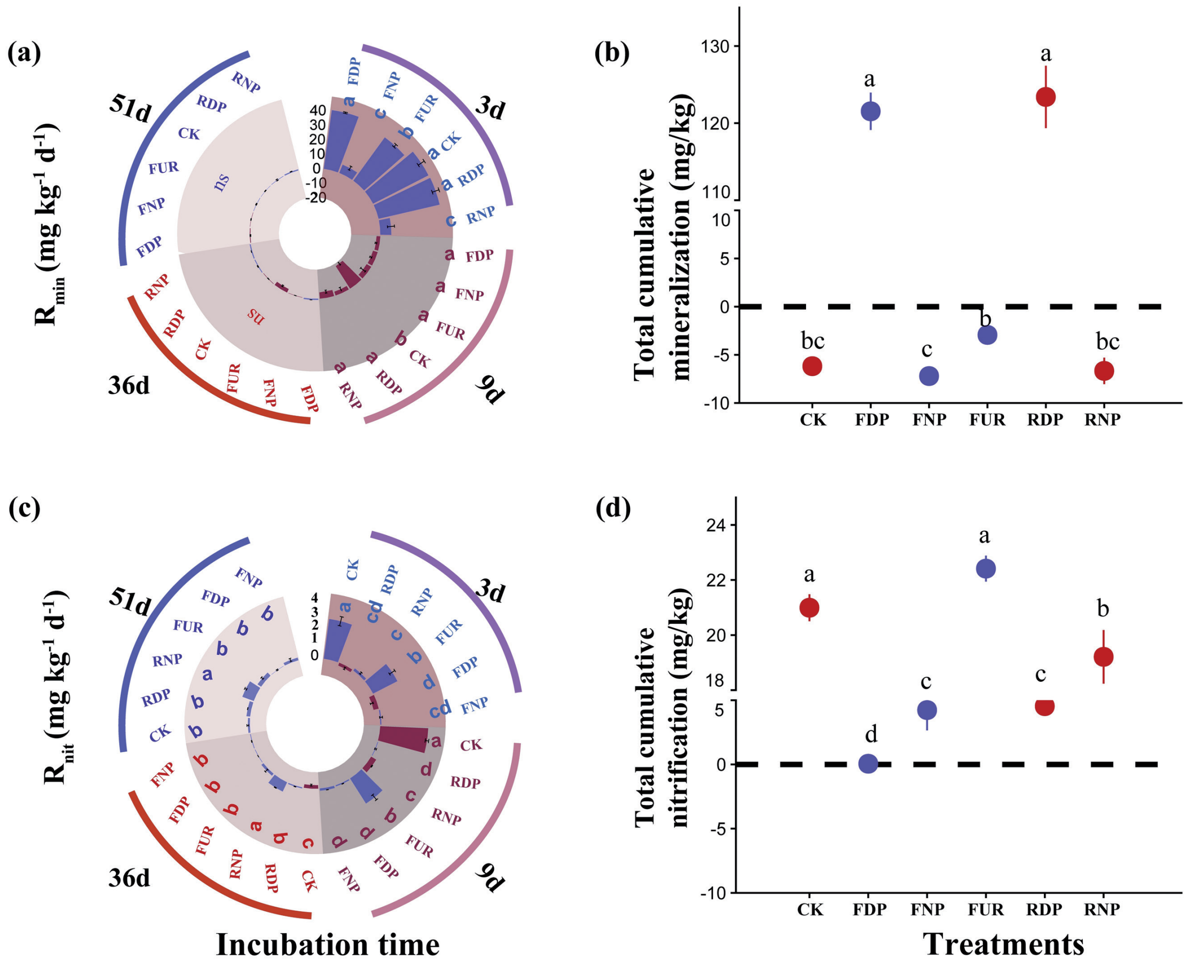

Fig. 3. Changes in net nitrogen mineralization rate (Rmin) and net nitrification rate (Rnit) of soil (mean ± SE, n = 3) under different incubation times. As well as, the effect of different incubation treatments on soil cumulative N mineralization and cumulative nitrification (mean ± SE, n = 3). Lowercase letters indicate significant differences (p<0.05) between treatments at the same incubation time.

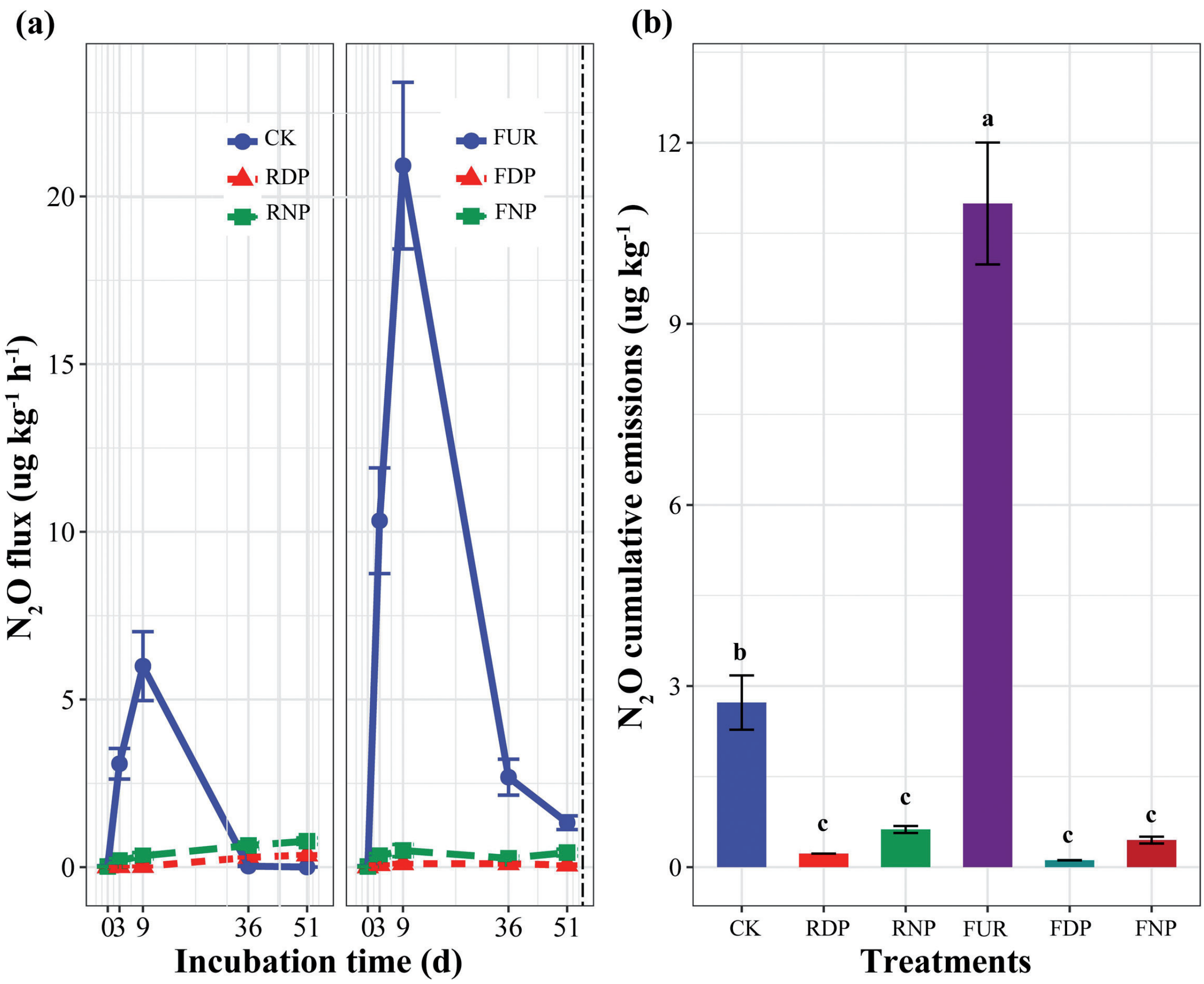

Fig. 4. Effect of different incubation treatments on soil N_2O emission flux and N_2O emission accumulation (mean ± SE, n = 3). Different lowercase letters indicate significant differences ($p<0.05$) between the incubation times.

Principal Component Analysis among Indicators

In the principal component analysis among the soil factors shown in Fig. 6, the first principal axis (Dim1) and the second principal axis (Dim2) explained 23.7% and 16.2%, respectively, and the cumulative explanation of the two principal axes was 39.9%. The contribution of each factor to the principal components was ranked as follows: TDN > NH_4^+-N > NO_3^--N > urease > N_2O > MBN > LA > PH > MBC > NAG > DOC > TOC > TN > SWC. The N_2O emission flux was linearly and positively correlated with MBN, MBC, NO_3^--N, and urease activity (Fig. 6).

Discussion

Effect of Urea with DMPP/NBPT on Soil Nitrogen Transformation

Ammonia nitrogen was not significantly decreased by the use of DMPP during continuous 25°C incubation; even despite this, inhibitors enlarged the pool of NO_3^--N in soil within the period of 3 to 51 days after fertilization (Fig. 2b). The possible explanation is that after urea application to soil, it is converted to NH_4^+-N through rapid hydrolysis by microbial urease, and NH_4^+-N is easily lost through volatilization or is oxidized to nitrate (NO_3^-) and leached out [29]. DMPP inhibits the transformation of NH_4^+-N to nitrite by suppressing the abundance of AOB-amoA, thus reducing the risk of NO_3^--N leaching and nitrogen loss [30-33], because the oxidation of ammonia nitrogen to NO_3^- (nitrification process) is controlled by ammonia monooxygenase (AMO), which is produced by nitrifying bacteria [ammonia oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA)] [34, 35]. As a whole, compared with N fertilizer alone (CK), we found that at the same N application rate, collaboration with DMPP (RDP, FDP) significantly inhibited the net nitrification rate of the soil (Fig. 3) and resulted in a lower NO_3^--N content (Fig. 2), especially in freeze-thaw treatments, implying that the application of a nitrification inhibitor (DMPP) significantly inhibited the nitrification process in the soil, reducing nitrogen losses in the fallow period (winter) and effectively improving soil N fertilizer utilization during the plant growing season in semi-arid areas [36, 37].

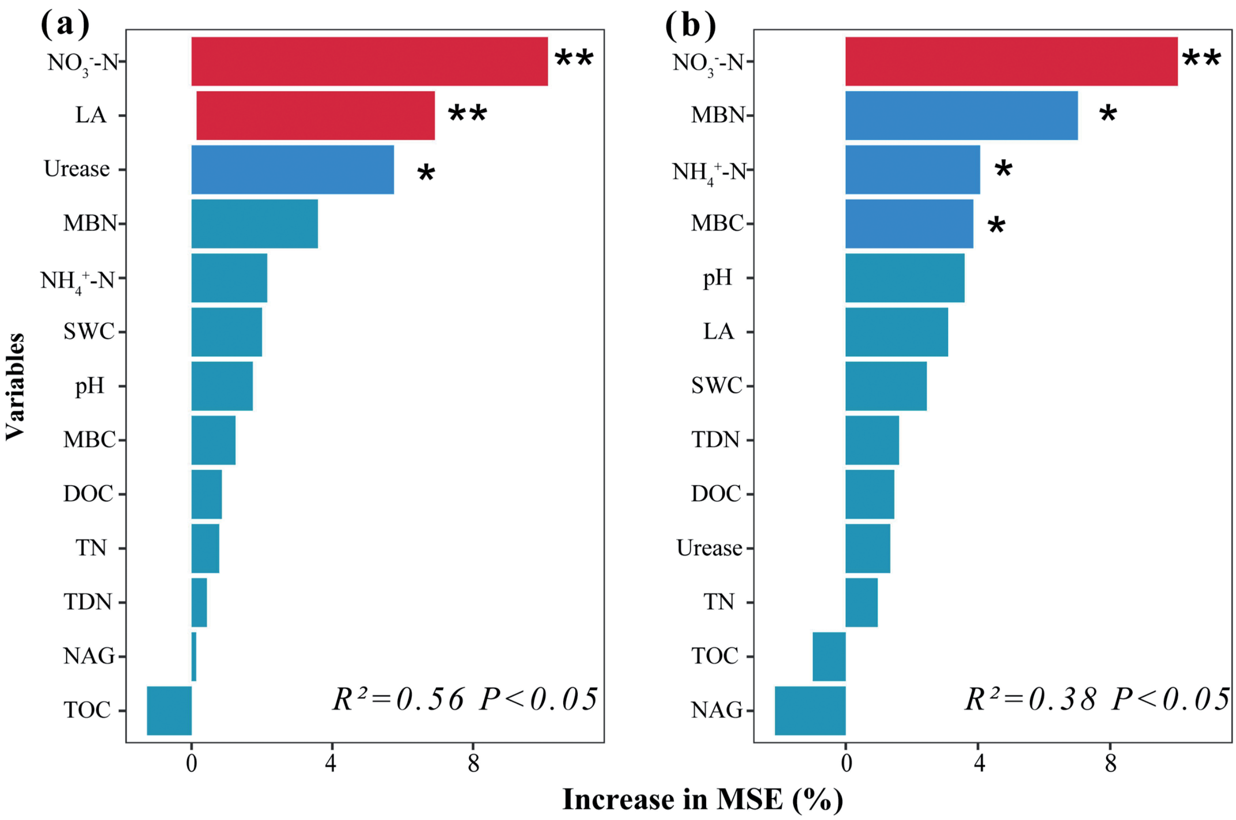

Fig. 5. Relative contribution of influencing factors to soil N_2O emission fluxes for all soil sampling dates under ambient 25°C incubation (a) and freeze-thaw incubation (b). The importance of predictor variables is estimated using the percentage increase in the mean squared error (MSE; %) from 100 runs of the random forest model. *p < 0.05, **p< 0.01.

The significantly lower NH_4^+-N concentration in the urea with NBPT (FNP and RNP) treatments compared to urea alone in the initial days indicates that the NBPT is inhibiting urea hydrolysis [38]. The decrease in soil NH_4^+-N concentration towards the end of the experiment in the NBPT treatments compared to DMPP addition (Fig. 2) could be due to the inhibition of the hydrolysis by NBPT as NBPT activity had been persisting; meanwhile, DMPP treatments could accelerate NH_4^+-N release even under freeze-thaw conditions. In addition, urea with NBPT application under freeze-thaw cycling incubation (FNP) and 25°C incubation (RNP) did not significantly change soil net mineralization rate (Fig. 3a); however, the net nitrification rate of soil gradually increased with increasing incubation time (Fig. 3c), and the cumulative nitrification was significantly different from that of CK (Fig. 3d). This could be explained by the action of NBPT slowing urea hydrolysis [38] through the inhibition of the urease enzyme in the soil and thus reducing the pool of exchangeable NH_4^+. This allowed more time for urea to diffuse into the soil.

Effect of Urea with DMPP/NBPT on Soil Nitrous Oxide Emissions

As shown in Fig. 5, N_2O emission fluxes and accumulations were largely influenced by the nitrogen treatments. DMPP suppressed the peak rates of soil nitrification and nitrous N_2O fluxes and attenuated cumulative soil nitrous oxide gas emissions by almost 70% (Fig. 4), almost completely suppressing N_2O emissions induced by urea treatment. This is consistent with the results of studies over the last decade confirming that the application of nitrification inhibitors significantly reduced N_2O emissions by inhibiting NH_4^+-N oxidation and delaying the nitrification process [8, 21]. Related studies have shown that functional genes encoding catalytic ammonia oxidases (AOA/AOB amoA genes) are commonly used as predictors of N_2O production and consumption [39-41]. We speculate that the present experiment may be because DMPP significantly reduced the transcript level of the AOB amoA gene and inhibited the growth and activity of ammonifying bacteria AOB in the soil, thereby inhibiting the oxidation of ammonia to nitrite and reducing nitrification by nitrifying bacteria, as well as reducing the substrate for the nitrifying bacterial denitrification and nitrification-coupled denitrification pathways, under the dual effect of DMPP on the autotrophic nitrification and denitrification processes. The N_2O emissions caused by urea were attenuated by the dual inhibition of autotrophic nitrification and denitrification processes by DMPP [42].

It has been shown that urea with NBPT can effectively suppress the peak N_2O emission flux caused by N fertilizer loss and reduce N loss [43,44]. Our results also confirmed this view, as urea with NBPT incubation suppressed

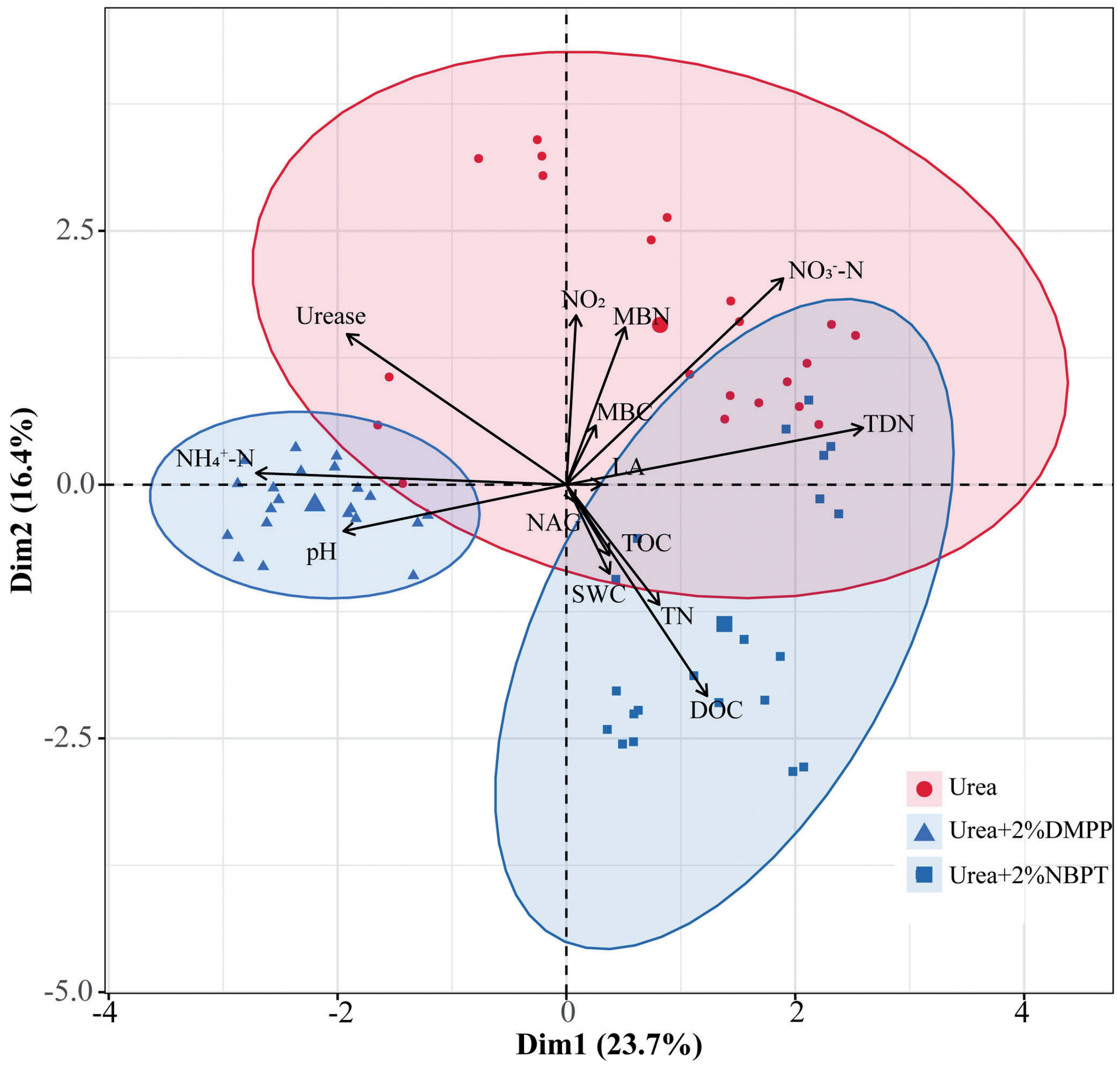

Fig. 6. Principal component analysis between soil variables.

the peak N_2O emission flux and significantly reduced cumulative N_2O emissions (Fig. 4). In contrast, some studies found that treatment with urea + NBPT (urease inhibitor) did not reduce direct N_2O emissions compared with urea alone [45]. Interestingly, some research has found that urea with the urease inhibitor NBPT has been shown to significantly reduce NH_3 emissions compared with urea alone [46]. As an indirect source of N_2O, the NH_3 loss from urea is also a net potential N_2O emission in the soil. More work is needed concerning NH_3 volatilization losses after NBPT application rather than focusing on urea hydrolysis only.

Effect of Freeze-Thaw Cycles on Soil N Transformation and Nitrous Oxide Emissions

With more frequent freeze-thaw, urea with DMPP under freeze-thaw conditions (FDP) resulted in a lower soil NO_3^--N content (Fig. 2b), a lower net nitrification rate (Fig. 3c), and significantly lower cumulative nitrification (Fig. 3d) compared to continuous 25°C incubation (RDP). In addition, the application of urea under freeze-thaw cycling incubation (FUR) resulted in a lower rate of decrease in the soil ammonium nitrogen content (Fig. 2a), a lower rate of increase in the nitrate nitrogen content, and a significantly lower rate of net soil nitrification within 9 days of incubation compared to CK, but there was no significant difference in cumulative nitrification (Fig. 3). The results proved that obvious soil freeze-thaw cycles in the freeze-thaw season could significantly affect nitrogen transformation and critical ecological processes in cold areas. These results also provide strong evidence that urea with a nitrification inhibitor (DMPP) can inhibit soil nitrification during the freeze-thaw period [20, 47] and that urea application alone did not suppress cumulative nitrification under freeze-thaw cycle incubation, although it suppressed the soil nitrification rates. The mechanism for this phenomenon is not yet clear, and other experiments are needed for further study.

This experiment showed that the most significant peak in N_2O emission flux was associated with the freeze-thaw cycle, and the freeze-thaw cycle incubation with urea alone resulted in a nearly 340% increase in the peak N_2O emission flux (Fig. 4), which confirms that the freeze-thaw cycle leads to increased N_2O emissions [48, 49]. We speculate that the disruption of soil aggregates during freezing, the release of their fixed nutrients and some reactive organic matter [50-52], and the death of soil microbes responsible for decomposition [53, 54] increase the soil matrix nutrients supplied for microbial use, thus promoting organic nitrogen mineralization and denitrification [55]. Most researchers concluded that denitrification is the dominant process responsible for N_2O emissions during the freeze-thaw cycle, especially during the soil thawing period [56]. It is also possible that during the freeze-thaw cycle incubation, water changes from liquid to solid and is fixed in the soil pore crevices, resulting in a decrease in the soil oxygen content and an increase in anaerobic microbial activity, which promotes denitrification [57] and can also be used to explain the random forest results under the freeze-thaw cycle incubation. NO_3^--N was the most important factor controlling the soil N_2O emission fluxes under freeze-thaw conditions (Fig. 5b).

Notably, we found that in the principal component analysis (Fig. 6), N_2O emission fluxes were linearly and positively correlated with MBN and MBC, while in the random forest regression analysis, MBN and MBC contributed significantly to N_2O gas emission fluxes under freeze-thaw conditions (Fig. 5). We speculate that this may be due to microbial death and decomposition resulting in elevated MBN and MBC in the freeze-thaw cycle, acting as nutrients and increasing the substrate used by denitrifying bacteria, resulting in enhanced denitrification [54]. During the initial freeze-thaw cycle, although microbial activity gradually increased, our incubation experiments were conducted in a confined indoor environment where the nutrient content of the soil matrix was not replenished and was gradually depleted, resulting in an increase in microbial activity at the beginning and a decrease in the later stages of incubation, so that N_2O emissions generally tended to increase first and then decrease. We not only confirmed that the freeze-thaw cycle promoted N_2O emissions, but we also found that N_2O emission fluxes did not peak when nitrification/urease inhibitors were applied under the freeze-thaw cycle, suggesting that N_2O emissions could also be suppressed by applying nitrification/urease inhibitors with urea under the freeze-thaw cycle.

Conclusion

In this study, we found that the freeze-thaw cycles inhibited the nitrification rate of the soil, which supported the hypothesis that the freeze-thaw changed the nitrogen transformation process of the irrigated desert soil. Furthermore, the freeze-thaw promoted N_2O emissions, and in response to the addition of the DMPP/NBPT inhibitor to the soil, the freeze-thaw induced soil N_2O emissions following urea application were mitigated due to inhibition. Our results support the hypothesis that the application of DMPP/NBPT inhibitors may lead to similar effects on soil N_2O emissions during the freeze-thaw period. Overall, N_2O emissions can be reduced by nitrogen dosing with DMPP/NBPT to reduce environmental pollution and nitrogen losses during the freeze-thaw period for agriculturally irrigated silt soil in the Minqin Oasis region.

Acknowledgments

This work was financially supported by the Science and Technology Program of Tibet Autonomous Region (XZ202201ZY0005N), Fundamental Research Funds for the Central Universities (lzujbky-2022-kb10), National Natural Sciences Foundation of China (NO. 41867013), Gansu Province Sciences Foundation (NO.20JR5RA074) and Gansu Province Key Research and Development Projects (NO.21CX6QA026). We also thank the anonymous reviewers for providing critical comments and suggestions that improved the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

References

1. CAMBOURIS A., ST LUCE M., ZEBARTH B., ZIADI N., GRANT C., PERRON I . Potato Response to Nitrogen Sources and Rates in an Irrigated Sandy Soil. Agronomy Journal, **108**, 391, **2016**.
2. STEVENS C.J. Nitrogen in the Environment. Science, **363**, 578, **2019**.
3. VENTEREA R.T., COULTER J.A., CLOUGH T.J. Nitrite Accumulation and Nitrogen Gas Production Increase with Decreasing Temperature in Urea-Amended Soils: Experiments and Modeling. Soil Biology and Biochemistry, **142**, 107727, **2020**.
4. HARTY M.A., FORRESTAL P.J., WATSON C.J., MCGEOUGH K.L., CAROLAN R., ELLIOT C., KROL D., LAUGHLIN R.J., RICHARDS K.G., LANIGAN G.J. Reducing Nitrous Oxide Emissions by Changing N Fertiliser Use from Calcium Ammonium Nitrate (CAN) to Urea Based Formulations. Science of The Total Environment, **563–564**, 576, **2016**.
5. WU D., ZHAO Z., HANA X., MENG F., WU W., ZHOU M., BRÜGGEMANN N., BOL R. Potential dual effect of nitrification inhibitor 3,4-dimethylpyrazole phosphate on nitrifier denitrification in the mitigation of peak N_2O emission events in North China Plain cropping systems. Soil Biology and Biochemistry, **121**, 147, **2018**.
6. HU H.W., CHEN D., HE J.Z. Microbial Regulation of Terrestrial Nitrous Oxide Formation: Understanding the Biological Pathways for Prediction of Emission Rates. FEMS Microbiology Reviews, **39**, 729, **2015**.
7. SILVA A.G.B., SEQUEIRA C.H., SERMARINI R.A., OTTO R. Urease Inhibitor NBPT on Ammonia Volatilization and Crop Productivity: A Meta-Analysis. Agronomy Journal, **109**, 1, **2017**.
8. WU D., ZHANG Y., DONG G., DU Z., WU W., CHADWICK D., BOL R. The Importance of Ammonia Volatilization in Estimating the Efficacy of Nitrification Inhibitors to Reduce

N_2O Emissions: A Global Meta-Analysis. Environmental Pollution, **271**, 116365, **2021**.

9. KESHAVARZ AFSHAR R., LIN R., MOHAMMED Y.A., CHEN C. Agronomic Effects of Urease and Nitrification Inhibitors on Ammonia Volatilization and Nitrogen Utilization in a Dryland Farming System: Field and Laboratory Investigation. Journal of Cleaner Production, **172**, 4130, **2018**.
10. DOUGHERTY W., COLLINS D., VAN ZWIETEN L., ROWLINGS D. Nitrification (DMPP) and Urease (NBPT) Inhibitors Had No Effect on Pasture Yield, Nitrous Oxide Emissions, or Nitrate Leaching under Irrigation in a Hot-Dry Climate. Soil Research, **54**, 675, **2016**.
11. XIAOYU H., LEI S., BAOKU Z., XINGZHU M., SHUANG W., SHUANGQUAN L., JINGHONG J., ENJUN K., SHAOJUN Q. Change in maize yield, N use efficiencies and climatic warming potential after urea combined with Nitrapyrin and NBPT during the growing season in a black soil, Soil and Tillage Research, **231**, 105721, **2023**.
12. NAIR D., BARAL K.R., ABALOS D., STROBEL B.W., PETERSEN S.O. Nitrate Leaching and Nitrous Oxide Emissions from Maize after Grass-Clover on a Coarse Sandy Soil: Mitigation Potentials of 3,4-Dimethylpyrazole Phosphate (DMPP). Journal of Environmental Management, **260**, 110165, **2020**.
13. YANG S., YUANCHUN Z., GUOPING W., XIAOFEI Y. Stimulation of nitrogen turnover due to nutrients release from aggregates affected by freeze-thaw in wetland soils. Physics and Chemistry of the Earth, **97**, 3, **2017**.
14. BROOKS P.D., GROGAN P., TEMPLER P.H., GROFFMAN P., ÖQUIST M.G., SCHIMEL J. Carbon and Nitrogen Cycling in Snow-Covered Environments. Geography Compass, **5**, 682, **2011**.
15. KING A.E., REZANEZHAD F., WAGNER-RIDDLE C. Evidence for microbial rather than aggregate origin of substrates fueling freeze-thaw induced N_2O emissions. Soil Biology and Biochemistry, **160**, 108352, **2021**.
16. QIANG F., JIAWEN Y., HENG L., TIANXIAO L., RENJIE H., DONG L., YI J. Effects of biochar amendment on nitrogen mineralization in black soil with different moisture contents under freeze-thaw cycles. Geoderma, **353**, 459, **2019**.
17. CONGREVES K.A., WAGNER-RIDDLE C., SI B.C., CLOUGH T.J. Nitrous oxide emissions and biogeochemical responses to soil freezing-thawing and drying-wetting. Soil Biology and Biochemistry, **117**, 5, **2018.**
18. ABALOS D., SMITH W.N., GRANT B.B., DRURY C.F., MACKELL S., WAGNER-RIDDLE C. Scenario Analysis of Fertilizer Management Practices for N2O Mitigation from Corn Systems in Canada. Science of The Total Environment, **573**, 356, **2016**.
19. WAGNER-RIDDLE C., CONGREVES K., ABALOS D., BERG A., BROWN S., AMBADAN J., GAO X., TENUTA M. Globally Important Nitrous Oxide Emissions from Croplands Induced by Freeze-Thaw Cycles. Nature Geoscience, **10**, 279, **2017**.
20. SONG Y., ZOU Y., WANG G., YU X. Altered Soil Carbon and Nitrogen Cycles Due to the Freeze-Thaw Effect: A Meta-Analysis. Soil Biology and Biochemistry, **109**, 35, **2017.**
21. RUSER R., SCHULZ R. The Effect of Nitrification Inhibitors on the Nitrous Oxide (N2O) Release from Agricultural Soils-a Review. Journal of Plant Nutrition and Soil Science, 1**78**, 171, **2015**.
22. DONG D., KOU Y., YANG W., CHEN G., XU H. Effects of Urease and Nitrification Inhibitors on Nitrous Oxide Emissions and Nitrifying/Denitrifying Microbial Communities in a Rainfed Maize Soil: A 6-Year Field Observation. Soil and Tillage Research, **180**, 82, **2018**.
23. FENG S., HUO Z., KANG S., TANG Z., WANG F. Groundwater simulation using a numerical model under different water resources management scenarios in an arid region of China. Environmental Earth Sciences, **62**, 961, **2011**.
24. JIANG X.J., LIU W., WANG E., ZHOU T., XIN P. Residual Plastic Mulch Fragments Effects on Soil Physical Properties and Water Flow Behavior in the Minqin Oasis, Northwestern China. Soil and Tillage Research, **166**, 100, **2017**.
25. CAMBOURIS A.N., ST LUCE M., ZEBARTH B., ZIADI N., GRANT C., PERRON I . Potato Response to Nitrogen Sources and Rates in an Irrigated Sandy Soil. Agronomy Journal, **108**, 391, **2016**.
26. SAIYA-CORK K.R., SINSABAUGH R.L., ZAK D.R. The Effects of Long Term Nitrogen Deposition on Extracellular Enzyme Activity in an Acer Saccharum Forest Soil. Soil Biology and Biochemistry, **34**, 1309, **2002**.
27. VANCE E.D., BROOKES P.C., JENKINSON D.S. An Extraction Method for Measuring Soil Microbial Biomass C. Soil Biology and Biochemistry, **19**, 703, **1987**.
28. SHAN Y., CHEN D., GUAN X., ZHENG S., CHEN H., WANG M., BAI Y. Seasonally Dependent Impacts of Grazing on Soil Nitrogen Mineralization and Linkages to Ecosystem Functioning in Inner Mongolia Grassland. Soil Biology and Biochemistry, **43,** 1943, **2011**.
29. FU Q., ABADIE M., BLAUD A., CARSWELL A., MISSELBROOK T.H., CLARK I.M., HIRSCH P.R. Effects of Urease and Nitrification Inhibitors on Soil N, Nitrifier Abundance and Activity in a Sandy Loam Soil. Biology and Fertility of Soils, **56**, 185, **2020**.
30. GILSANZ C., BAEZ D., MISSELBROOK T.H., DHANOA M.S., CARDENAS L.M. Development of Emission Factors and Efficiency of Two Nitrification Inhibitors, DCD and DMPP. Agriculture Ecosystems & Environment, **216**, 1, **2016**,
31. SHI X., HU H.W., ZHU-BARKER X., HAYDEN H., WANG J., SUTER H., CHEN D., HE J.Z. Nitrifier-Induced Denitrification Is an Important Source of Soil Nitrous Oxide and Can Be Inhibited by a Nitrification Inhibitor 3,4-Dimethylpyrazole Phosphate. Environmental Microbiology, **19**, 4851, **2017**.
32. CHEN H., YIN C., FAN X., YE M., PENG H., LI T., ZHAO Y., WAKELI S.A., CHU G., LIANG Y. Reduction of N_2O Emission by Biochar and/or 3,4-Dimethylpyrazole Phosphate (DMPP) Is Closely Linked to Soil Ammonia Oxidizing Bacteria and nosZI-N_2O Reducer Populationsyy. Science of the Total Environment, **694**, 133658, **2019**.
33. VILARRASA-NOGUE M., TEIRA-ESMATGES M.R., PASCUAL M., VILLAR J.M., RUFAT J. Effect of N Dose, Fertilisation Duration and Application of a Nitrification Inhibitor on GHG Emissions from a Peach Orchard. Science of the Total Environment, **699**, 134042, **2020**.
34. LEVY-BOOTH D.J., PRESCOTT C.E., GRAYSTON S.J. Microbial Functional Genes Involved in Nitrogen Fixation, Nitrification and Denitrification in Forest Ecosystems. Soil Biology and Biochemistry, **75**, 11, **2014**.
35. ZHOU L.-J., HAN P., ZHAO M., YU Y., SUN D., HOU L., LIU M., ZHAO Q., TANG X., KLÜMPER U., GU J.D., MEN Y., WU Q.L. Biotransformation of lincomycin and fluoroquinolone antibiotics by the ammonia oxidizers AOA, AOB and comammox: A comparison of removal, pathways, and mechanisms. Water Research, **196**, 117003, **2021**.
36. MIGLIORATI M.A., PARTON W.J., BELL M.J., WANG W., GRACE P.R. Soybean fallow and nitrification inhibitors: Strategies to reduce N_2O emission intensities and N losses in Australian sugarcane cropping systems. Agriculture, Ecosystems & Environment, **306**, 107150, **2021**.

37.QIAOGANG Y., JUNWEI M., WANCHUN S., PING Z., HUI L., CHANGHUAN F., JIANRONG F. Evaluations of the DMPP on Organic and Inorganic Nitrogen Mineralization and Plant Heavy Metals Absorption. Geoderma, **312**, 45, **2018**.
38.DEMPSEY R.J., SLATON N.A., ROBERTS T.L., NORMAN R.J. Rice grain yield and nitrogen uptake as affected by urea amendment and rainfall timing. Agronomy Journal, **109**, 2966, **2017**.
39.GAO D., ZHANG L., LIU J., PENG B., FAN Z., DAI W., JIANG P., BAI E. Responses of Terrestrial Nitrogen Pools and Dynamics to Different Patterns of Freeze-Thaw Cycle: A Meta-Analysis. Global Change Biology, **24**, 2377, **2018**.
40. ZHANG H., FANG Y., CHEN Y., LI Y., WU J., CAI Y., SCOTT X.C. Enhanced soil potential N2O emissions by land-use change are linked to AOB-amoA and nirK gene abundances and denitrifying enzyme activity in subtropics. Science of The Total Environment, **850**, 158032, **2022**.
41.GAO J., XIE Y., JIN H., LIU Y., BAI X., MA D., ZHU Y., WANG C., GUO T. Nitrous Oxide Emission and Denitrifier Abundance in Two Agricultural Soils Amended with Crop Residues and Urea in the North China Plain. PLoS One, **11**, e0154773, **2016**.
42.ZHU G., JU X., ZHANG J., MÜLLER C., REES R.M., THORMAN R.E., SYLVESTER-BRADLEY R. Effects of the Nitrification Inhibitor DMPP (3,4-Dimethylpyrazole Phosphate) on Gross N Transformation Rates and N_2O Emissions. Biology And Fertility Of Soils, **55**, 603, **2019**.
43.DING W.X., CHEN Z.M., YU H.Y., LUO J.F., YOO G.Y., XIANG J., ZHANG H.J., YUAN, J.J. Nitrous Oxide Emission and Nitrogen Use Efficiency in Response to Nitrophosphate, N-(n-Butyl) Thiophosphoric Triamide and Dicyandiamide of a Wheat Cultivated Soil under Sub-Humid Monsoon Conditions. Biogeosciences, **12**, 803, **2015**.
44.WANG H., MA S., SHAO G., DITTERT K. Use of Urease and Nitrification Inhibitors to Decrease Yield-Scaled N_2O Emissions from Winter Wheat and Oilseed Rape Fields: A Two-Year Field Experiment. Agriculture, Ecosystems & Environment, **319**, 107552, **2021**.
45.KROL D.J., FORRESTAL P.J., WALL D., LANIGAN G.J., SANZ-GOMEZ J., RICHARDS K.G. Nitrogen fertilisers with urease inhibitors reduce nitrous oxide and ammonia losses, while retaining yield in temperate grassland. Science of the Total Environment, **725**, 138329, **2020**.
46.WATSON C.J., LAUGHLIN R.J., MCGEOUGH K.L. Modification of nitrogen fertilisers using inhibitors: opportunities and potentials for improving nitrogen use efficiency. In: Proceedings-International Fertiliser Society (No. 658). International Fertiliser Society, ISSN **1466–1314,** pp. 1, **2009.**
47.URAKAWA R., SHIBATA H., KUROIWA M., INAGAKI Y., TATENO R., HISHI T., FUKUZAWA K., HIRAI K., H TODA., OYANAGI N., NAKATA M., NAKANISHI A., FUKUSHIMA K, ENOKI T., SUWA Y. Effects of Freeze–Thaw Cycles Resulting from Winter Climate Change on Soil Nitrogen Cycling in Ten Temperate Forest Ecosystems throughout the Japanese Archipelago. Soil Biology and Biochemistry, **74**, 82, **2014**.
48. PENG B., SUN J., LIU J., DAI W., SUN L., PEI G., GAO D., WANG C., JIANG P., BAI E. N_2O Emission from a Temperate Forest Soil during the Freeze-Thaw Period: A Mesocosm Study. Science of The Total Environment, **648**, 350, **2019**.
49. EJACK L., WHALEN J.K. Freeze-Thaw Cycles Release Nitrous Oxide Produced in Frozen Agricultural Soils. Biology And Fertility Of Soils, **57**, 389, **2021**.
50. FENG, Z., LI, Z., LI, P., XIAO, L. Effects of freeze-thaw cycles and soil moisture content on soil available micronutrients on aggregate scale in natural grassland and Chinese pine forestland on the Loess Plateau, China. Journal Of Soils And Sediments, **20**, 4023, **2020**.
51. BAILEY V.L., PRIES C.H., LAJTHA K. What Do We Know about Soil Carbon Destabilization? Environmental Research Letters, **14**, 083004, **2019**.
52. ROONEY E.C., BAILEY V.L., PATEL K.F., DRAGILA M., BATTU A.K., BUCHKO A.C., GALLO A.C., HATTEN J., POSSINGER A.R., QAFOKU O., RENO L.R., SANCLEMENT S.M, VARGA T., LYBRAND R.A. Soil Pore Network Response to Freeze-Thaw Cycles in Permafrost Aggregates. Geoderma, **411**, 115674, **2022**.
53. NAN J., YINGHUA J., LULU T., XIAODONG C., WENTAO S., LIJUN C. Modification of the composition of dissolved nitrogen forms, nitrogen transformation processes, and diversity of bacterial communities by freeze-thaw events in temperate soils, Pedobiologia, **71**, 41, **2018**.
54. SAWICKA J.E., ROBADOR A., HUBERT C., JØRGENSEN B.B., BRÜCHERT V. Effects of Freeze-Thaw Cycles on Anaerobic Microbial Processes in an Arctic Intertidal Mud Flat. ISME Journal, **4**, 585, **2010**.
55. QIU L., GOU X., KONG Y., TU F., PENG X., XU L., ZHOU S., HUANG C., CHEN Y., LIU L., TU L. Nitrogen addition stimulates N_2O emissions via changes in denitrification community composition in a subtropical nitrogen-rich forest. Journal of Environmental Management, **348**, 19274, **2023**.
56. SONG Y., ZOU Y.C., WANG G.P., YU X.F. Altered soil carbon and nitrogen cycles due to the freeze-thaw effect: a meta-analysis. Soil Biology and Biochemistry, **109**, 35. **2017**.
57.MØRKVED P.T., DÖRSCH P., HENRIKSEN T.M., BAKKEN L.R. N_2O Emissions and Product Ratios of Nitrification and Denitrification as Affected by Freezing and Thawing. Soil Biology and Biochemistry, **38**, 3411, **2006**.