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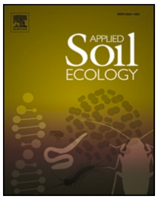

Combining straw amendment and nitrification inhibitor to mitigate soil N losses during cooling-warming and freeze-thaw cycles

Hao Chen [a,b], Sergey Blagodatsky [a,c,*], Christoph Rosinger [d], Rüdiger Reichel [e], Bo Li [f], Amit Kumar [g], Steffen Rothardt [h], Nicolas Brüggemann [e], Henning Kage [h], Michael Bonkowski [a]

[a] *University of Cologne, Department of Biology, Institute of Zoology, Terrestrial Ecology, Germany*
[b] *Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Theodor-Echtermeyer-Weg 1, 14979 Grossbeeren, Germany*
[c] *Institute of Meteorology and Climate Research, Department of Atmospheric Environmental Research (IMK-IFU), Karlsruhe Institute of Technology (KIT), Germany*
[d] *Institute of Agronomy, Department of Agricultural Sciences, BOKU University, 3430 Tulln an der Donau, Austria*
[e] *Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences, Agrosphere (IBG-3), Jülich, Germany*
[f] *College of Natural Resources and Environment, South China Agricultural University, Guangzhou, Guangdong 510642, PR China*
[g] *Department of Biology, College of Science, United Arab Emirates University, Al Ain, United Arab Emirates*
[h] *Agronomy and Crop Science, Institute of Crop Science and Plant Breeding, Christian-Albrechts-University, Kiel, Germany*

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ABSTRACT

Post-harvest losses of nitrogen (N) from agricultural fields causes environmental damage due to N leaching and greenhouse gas emissions. Two potential strategies to mitigate these losses are amending soil with straw and applying nitrification inhibitors (NIs). Combining these strategies could have a synergistic effect, reducing nitrification and promoting the long-term microbial N immobilization. However, the effect of seasonal temperature fluctuations on N losses – when straw and NIs are applied together – is poorly understood. To investigate this, we conducted a 3-month mesocosm experiment examining the impact of wheat straw amendment and NI on soil N losses via N_2O emissions and leaching, depending on temperature variation (freezing-thawing vs cooling-warming), with and without mineral N fertilization. We observed an increase in N_2O emissions immediately after applying the straw and N fertilizer, which was reduced by 58% with NI application. Both freezing-thawing and mild cooling-warming led to increase in net N mineralization, causing a second N_2O emission peak. However, adding straw reduced N leaching by 70%, thus mitigating total N losses. The positive overall effect of the combined application of straw and NI resulted from the selective actions of each measure: the N immobilization induced by straw reduced leaching, while NI application decreased N_2O emissions, which were stimulated by the addition of straw. This “double strike” approach was particularly efficient under strong temperature variations. Our findings have practical importance and should be considered when attempting to mitigate N losses through the application of straw and NI.

1. Introduction

Substantial nitrogen (N) losses occur in agricultural soils between crop cultivation phases, especially during winter, when fluctuating soil moisture and temperature accelerate N release before it can benefit the succeeding crop generation (Cookson et al., 2002; Henke et al., 2008; Li et al., 2021). These fertilizer-derived N losses negatively affect the global climate and environmental quality (Kumar et al., 2020; Rothardt and Kage, 2024; Tian et al., 2020). While many studies have examined long-term N transformations and losses under constant soil incubation temperatures (Abdalla et al., 2009; Cookson et al., 2007; Dai et al., 2020) or after short-term freeze-thaw stress (Matzner and Borken, 2008; Rosinger and Bonkowski, 2021; Rosinger et al., 2022a), experiments mimicking seasonal N transformations in agricultural soils remain scarce (Sieling and Kage, 2006).

Nitrification inhibitors (NIs) are widely used to curb N losses and enhance crop N use efficiency (Wu et al., 2017). These compounds inhibit ammonia-oxidizing bacteria, preventing the oxidation of ammonium (NH_4^+) to nitrite (NO_2) (Subbarao et al., 2006; Zerulla et al., 2001). Although NIs are known to reduce N_2O emissions, their

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* Corresponding author at: Institute of Meteorology and Climate Research, Department of Atmospheric Environmental Research (IMK-IFU), Karlsruhe Institute of Technology (KIT), Germany.
E-mail address: sergey.blagodatskiy@kit.edu (S. Blagodatsky).

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effectiveness depends strongly on soil temperature, pH and fertilization level (Qiao et al., 2015; Ruser and Schulz, 2015; Norton and Ouyang, 2019). Warmer soil conditions, for example, shorten their inhibitory duration and reduce their efficiency (Irigoyen et al., 2003; McGeough et al., 2016).

Incorporating crop straw as a high-carbon amendment (HCA) is among the oldest and most cost-effective practices worldwide to reduce soil N losses (Diacono and Montemurro, 2011; Norton and Ouyang, 2019; Xia et al., 2018). By supplying bioavailable carbon (C), straw stimulates microbial growth and microbial N immobilization in biomass (Rosinger et al., 2022b), as shown in both incubation (Congreves et al., 2013a; Chen et al., 2023; Reichel et al., 2022; Zavalloni et al., 2011) and field studies (Congreves et al., 2013b; Török et al., 2014). However, its effects on soil N_2O emissions are inconsistent - ranging from decreases (Rothardt et al., 2021; Shan and Yan, 2013; Yao et al., 2017) to neutral (John et al., 2020; Malhi and Lemke, 2007) or even increases (Li et al., 2013; Xia et al., 2018). The variable outcomes reflect interactions between soil properties, moisture, and fertilization practices (Chen et al., 2013; Yu et al., 2019), indicating that the mechanisms underlying straw-mediated impacts on N_2O emissions are complex (Wu et al., 2020).

While straw amendment often reduces N leaching via microbial N immobilization, it can simultaneously enhance N_2O emission through denitrification. Moisture and oxygen availability are key to regulating this balance and straw decomposition acts as an additional consumer of O_2. Therefore, seasonal cooling-warming and freezing-thawing cycles are likely to alter the effects of straw addition on N cycling, with strong temperature fluctuations exerting a larger impact (Pelster et al., 2013). Under such conditions, NI can suppress nitrification and associated N_2O production (Chen et al., 2021), but may also limit NO_3^- availability for denitrification. Thus, combining NI with straw could provide a complementary strategy to mitigate N losses, although this synergy remains experimentally unverified. It is still uncertain whether NI and HCA mitigation approaches can be effectively combined under fluctuating and below freezing soil temperatures. To this end, we conducted a mesocosm experiment to study N dynamics between crop cultivation phases. Following straw incorporation, soils received mineral N fertilizer and the NI Piadin® in a multifactorial design. The mesocosms were subjected to two temperature regimes: cooling-warming and freezing-thawing. Over a period of 71 days, we quantified N losses (as N_2O emissions and leachate) and monitored microbial N cycling, including microbial biomass and the abundances of functional genes.

We hypothesized that i) the combined application of a nitrification inhibitor (NI) and straw would have additive effects in reducing N losses, with NI mitigating the stimulation of N_2O emissions caused by the joint effect of fertilizer-derived N and straw-derived C; and ii) freezing-thawing cycles would trigger substantial N_2O emissions and N leaching losses, whereas cooling-warming cycles alone would not. By integrating multiple analytical approaches previously applied in separate studies (e.g., Chen et al., 2021; Barrena et al., 2017), this study aims to test these hypotheses and generate new insights into N dynamics. Specifically, it enables the joint evaluation of N_2O flux dynamics, mineral N forms, and microbial functional genes governing nitrification and denitrification.

2. Materials and methods

2.1. Soil and experiment design

The agricultural soil used in this study was obtained from the Experimental Farm Hohenschulen, Kiel University, Germany (54°18′N, 9°58′E) in June 2019. The soil is classified as Luvisol, with the following properties: pH 6.5, total organic C 1.07%, total N 0.11%, sand 58%, silt 29%, clay 13%. The soil was sieved (1 cm mesh size) and homogenized before the experiment.

A mesocosm experiment was set up in a climate chamber with a factorial design ($n = 7$) to simulate seasonal temperature variation during the period between vegetation phases: (i) a conditioning phase simulating the incorporation of crop residues (−/+ wheat straw) after harvest without (−N) and with nitrogen (+N) fertilization after 7 days, without (-NI) and with (+NI) nitrification inhibitor application (Phase 1, days 0–28), and subsequently subjecting fertilized (+N) mesocosms to (ii) two different winter temperature treatments (Phase 2, days 29–71).

Mesocosms (22 cm height, 24 cm diameter; 10 L volume) were equipped with a central drainage tube (1 cm diameter) at the bottom to collect leachate. Each mesocosms was filled to a depth of 13 cm with 8 kg dry-weight soil with a gravimetric water content of 9%, corresponding to a bulk density of 1.2 g cm^{-3}. Into half of the mesocosms, wheat straw (41.8% C, 0.84% N, pieces of 2 to 3 cm) was mixed into the soil at a rate of 2.4 g C kg^{-1} (eq. 14.4 t ha^{-1} wheat straw amendment). One week after pre-incubating all 84 mesocosms, fertilization was applied to +N treatments as 73 mg N kg^{-1} soil ($(NH_4)_2SO_4$), equivalent to 250 kg N ha^{-1}. The nitrification inhibitor Piadin® (SKW Piesteritz, Germany), containing the active compounds 1H-1,2,4-triazole (3.1%) and 3-methylpyrazole (1.6%), was applied to +NI treatments. Piadin was applied twice: together with N fertilizer on day 1 (equivalent to 7 L ha^{-1}) and again on day 62 (6 L ha^{-1}), corresponding to a total application rate of 13 L ha^{-1}. Fertilizer and inhibitor were applied as aqueous solutions to the soil surface.

During Phase 1, the average daily temperature was kept at approximately 19 °C. In Phase 2, temperature was reduced to 7 °C from days 29 to 58. Subsequently, two temperature treatments were applied. In the **+N cooling treatment**, temperature increased gradually to 21 °C between days 58 and 71. In the **+N freezing treatment**, soils were first frozen at −20 °C (days 58–60) and then thawed at 7 °C (days 60–62), after which temperature increased to 21 °C until day 71 (Fig. 1). Unfertilized mesocosms followed the same temperature regime as the +N cooling treatment.

Mesocosms were irrigated with tap water every second day to maintain 50% water-holding capacity throughout the experiment, with water loss monitored by weighing.

2.2. Measurements

Gas samples for determining net N_2O, and CO_2 emissions were collected at 13 regular intervals, with 4 replicates per treatment, using the static-opaque chamber method (Dobbie et al., 1999). Chambers consisted of PVC tubes (20 cm diameter, 55 cm height) closed with an airtight lid fitted with tubing and a three-way cock for gas sampling. Chambers were inserted to a depth of 3 cm into the soil surface of each mesocosm to ensure gas tightness. During each sampling, three 20 mL headspace samples were withdrawn at 30, 60 and 90 min after chamber placement. As controls, five ambient air samples were collected per sampling event, and the average N_2O and CO_2 concentrations were used as baselines. All samplings were performed between 11 a.m. and 1 p.m.

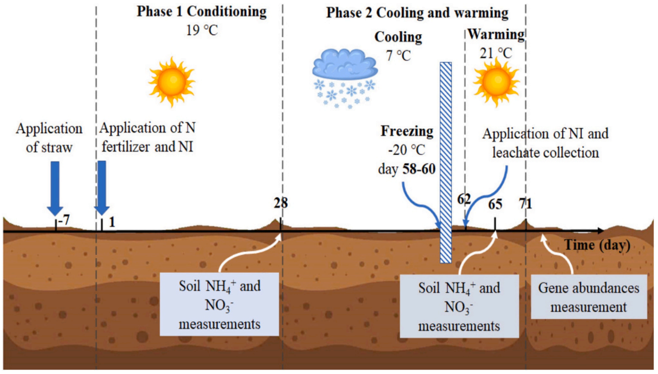

Fig. 1. Schematic presentation of the experimental setup. Details on application rates of straw and fertilizer, as well as nitrification inhibitor (NI), can be found in the "Material and methods" section.

Soil NH_4^+-N and NO_3^--N were determined from approximately 30 g of soil sampled from three opposite locations in each mesocosm using a 2 cm diameter corer at 0–10 cm depth. Samples were collected at the end of Phase 1 (day 28), and after soil warming (day 65). For functional gene abundance analysis samples were immediately frozen at −20 °C (Fig. 1). Soil NH_4^+-N and NO_3^--N contents were extracted with 20 mL 0.01 M $CaCl_2$ from 5 g fresh wt soil after shaking (30 min). After centrifugation (5 min, 4500 rpm) and filtering (Whatman 595 filter paper) the supernatant, and determined using ion-selective electrodes for NH_4^+ (ELIT 8051) and NO_3^- (ELIT 8021, Nico 2000 Ltd., UK). Soil mineral N (N_{min}) was calculated as the sum of NH_4^+-N and NO_3^--N. Soil pH (multi 340i, WTW GmbH, Weilheim, Germany) was determined according to ISO 10390 guidelines (ISO, 2005) from a suspension of 5 g fresh wt soil in 1 M KCl at 1:5 (*w*/*v*) after shaking for 2 h.

To collect the leachate, the mesocosms were watered with 600 ml of water on day 62, the second day of the warming phase. The volumes of leachate were recorded, and the concentrations of NO_3^--N and NH_4^+-N were measured using ion-sensitive electrodes, as described above.

Functional marker genes involved in nitrification (AOB and AOA *amoA*) and denitrification (bacterial *nirK*, *nirS*, *nosZ*), were amplified by quantitative real-time PCR (qPCR) using a 2× qPCR Master Mix (Nanjing Novizan Biotechnology Co., China) on a real-time PCR detection platform operated by the commercial laboratory. All nucleic acid extracts were diluted ten-fold with nuclease-free water prior to amplification to decrease the impact of PCR inhibition and increase reaction sensitivity. The qPCR reaction mixtures contained 10 μL of 2× qPCR Mix, 0.4 μL of 10 μM each of forward and reverse primer, 1.5 μL of diluted DNA template, and nuclease-free water to a final volume of 20 μL. Thermal cycling conditions consisted of 95 °C for 5 min, followed by 45 cycles of 95 °C for 15 s, 55 °C for 30 s, and 72 °C for 30 s, with a melting curve analysis were performed at the end of each run. The qPCR data presented in this study were derived from independent extractions of six replicates. Standards ranging from 10^{10} to 10^5 gene copies μL^{-1} were prepared from plasmids with insertions of target gene fragments. The amplification efficiencies and R^2 values generated from these standard curves met the accepted criteria for all genes.

2.3. Calculations and statistical analysis

The N_2O emission factors were calculated as the amount of net N_2O emissions in the fertilized treatments minus the net emissions in the unfertilized treatment (background N_2O emissions) as the percentage of the fertilizer N applied for the period between gas samplings. The global warming potential (GWP, g CO_2 equivalent/kg soil) of total greenhouse gas emissions was calculated with CO_2 as reference gas, where an increase or reduction in emissions of N_2O were converted into 'CO_2-equivalents' by means of their GWPs (IPCC, 2014). Based on 6 IPCC assessment report (Forster et al., 2021), Eq. (1) was used to calculate the GWP:

$$\begin{aligned}\text{GWP}\ \left(\text{g CO}_2\ \text{equivalent kg}^{-1}\ \text{soil}\right) &= \text{CO}_2\ \left(\text{g CO}_2\ \text{kg}^{-1}\ \text{soil}\right)\\ &+ 273\times \text{N}_2\text{O}\ \left(\text{g N}_2\text{O kg}^{-1}\ \text{soil}\right)\end{aligned} \tag{1}$$

All statistical analyses were performed in R 3.6.3 (R Core Team, 2020) and graphs were prepared in Origin Pro 8.1 (Origin Lab, Northampton, MA, USA). Treatment effects were analysed using analyses of variance (ANOVA) and a priori contrasts (as detailed in the supplementary information). Linear regression analyses were used to evaluate the relationships of the parameters from soil, microorganisms, and N_2O emission.

Structural equation modeling (SEM; 'lavaan' package; Rosseel, 2012), was applied to identify the relationships between N fertilization, straw application, soil mineral N content and N_2O emissions during the conditioning phase of the experiment. It should be noted that SEM evaluates hypothesized relationships among variables based on covariance structures and does not establish causal relationships. Prior to the SEM procedure, principal component analysis (PCA) – as implemented in the vegan package (Oksanen et al., 2025) – was conducted to identify and remove variables with collinearity. The hypothetical relationships among the variables in the models were constructed based on the results of correlation analyses (Fig. S1). The best fitting model was selected by step-wise removal of non-significant paths. The data were square root-transformed before the SEM analysis considering nondimensional expression.

The criteria for evaluation of the structural equation model fit, such as the Chi-square/degree values (CHI/DF), goodness-of-fit index (GFI) and standardized root mean square residual (SRMR) were adopted according to Shen et al. (2021).

3. Results

Nitrous oxide (N_2O) emissions showed two distinct peaks: the first occurred at the start of the conditioning phase, strongly enhanced by N fertilizer application, and the second followed soil cooling or freezing. Together, these peaks accounted for approximately 90% of total cumulative N_2O emissions, with the first and second peaks contributing 33% and 56%, respectively (Fig. 2). Consequently, N_2O emissions and related soil parameters are presented separately for the conditioning phase (Section 3.1) and winter temperature treatment phase (Section 3.2).

3.1. N immobilization and N_2O emissions during the Phase 1 (conditioning)

Straw amendment reduced total soil mineral N (N_{min}) by about 50% (Fig. 3E, $p < 0.001$), independent of N fertilization. NI application increased N_{min} by 32% in unfertilized (−N) treatments without straw (Fig. 3E, $p < 0.05$). NO_3^--N comprised 67–92% of N_{min}, and straw amendment strongly reduced NO_3^--N levels (Fig. 3C), while NH_4^+-N slightly increased in unfertilized (−N) straw addition treatments (+14.6%, $p < 0.001$; Fig. 3A). NI significantly increased NH_4^+-N only in fertilized mesocosms without straw (+39.6%, $p < 0.001$), and raised NO_3^--N by 38.6% in unfertilized non-straw treatments ($p < 0.05$).

In Phase 1, straw addition amplified the peak N_2O emissions 1.5 and

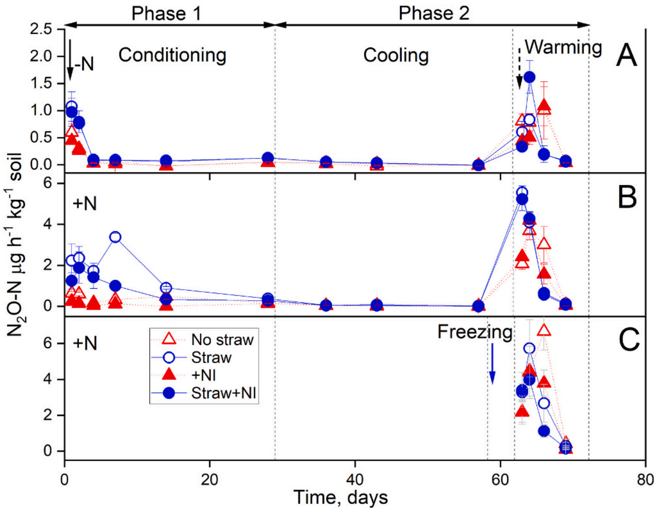

Fig. 2. Dynamics of N_2O emission under different treatments during the incubation experiment. Panel A shows the unfertilized treatments (−N); panel B shows the +N treatments without freezing-thawing; and panel C shows the +N treatments with freezing-thawing. Please note the different Y-axis scale for panel A. The means ±1SE ($n = 4$) are shown, the dash lines separate the different phases of experiment, the black solid arrow indicates application of N fertilizer and NI, the dash arrow indicates application of NI only, blue arrow indicates the freezing event.

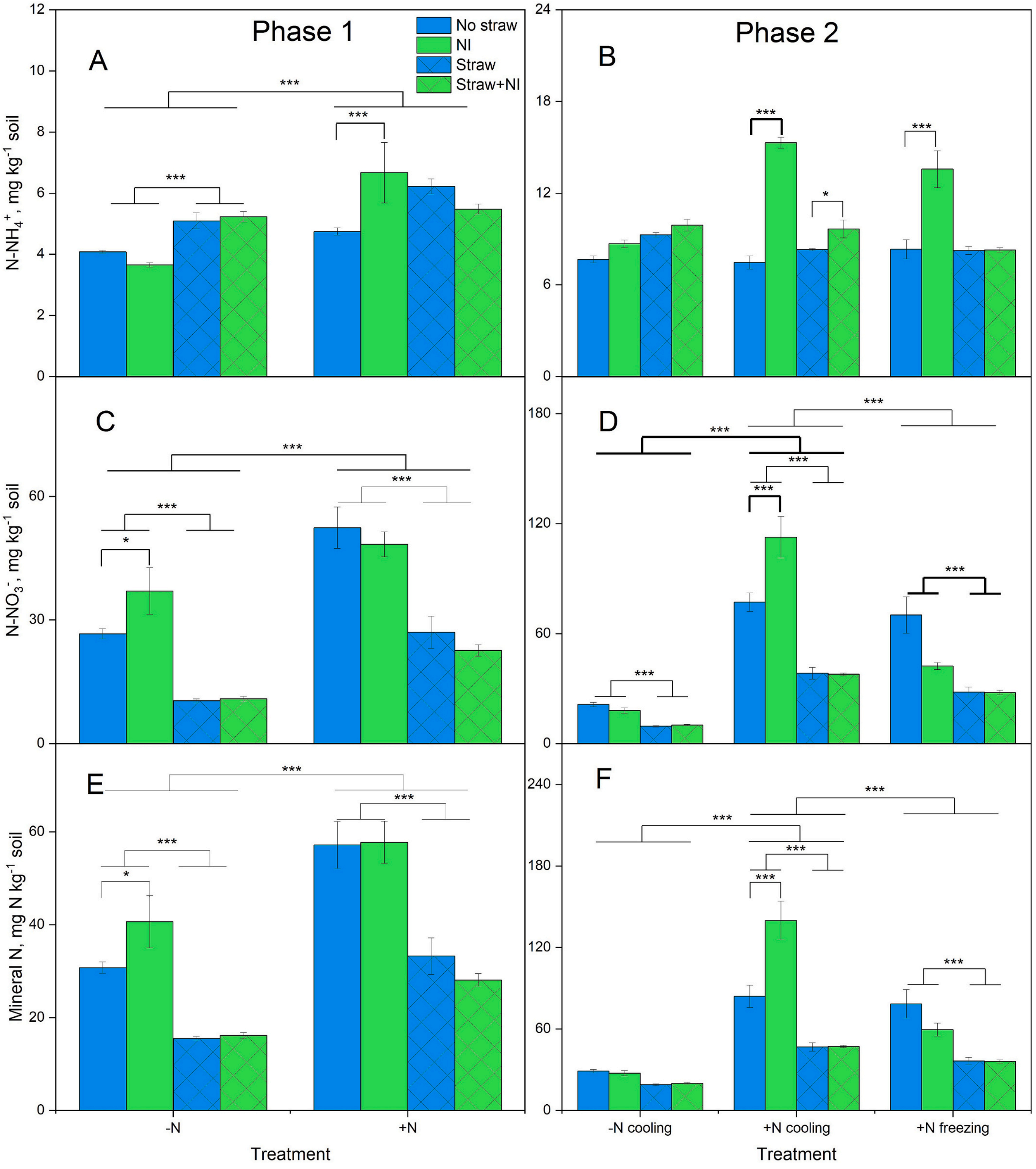

Fig. 3. Soil NH_4, NO_3 and mineral N contents as affected by the application of straw and NI, fertilization, and temperature manipulations, broken down by experiment phase (graphs A, C, E and B, D, F, respectively) in unfertilized (−N) and fertilized (+N) soil before and after cooling or freezing. The means ±1SE ($n = 7$) and difference between treatments based on ANOVA with contrasts, are presented with the following indications of statistical significance: *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

5.5 fold in −N and +N treatments, respectively (Fig. 2). Overall, N fertilization increased cumulative N_2O emissions 6.5-fold (Fig. 4B), while straw addition increased it 5-fold in Phase 1. NI reduced N_2O emissions by 59% in fertilized mesocosms ($p < 0.01$; Fig. 4; Table S1).

At the end of Phase 1, cumulative N_2O emissions correlated positively with residual soil NO_3^--N content in straw addition treatments ($p < 0.001$; Fig. S1A). However, this relationship turned negative in fertilized soils (+N) ($p < 0.001$; Fig. S1B), reflecting the strong link between NO_3^--N and denitrification. The strongest negative correlation between N_2O and NO_3^--N was found in fertilized treatments without NI

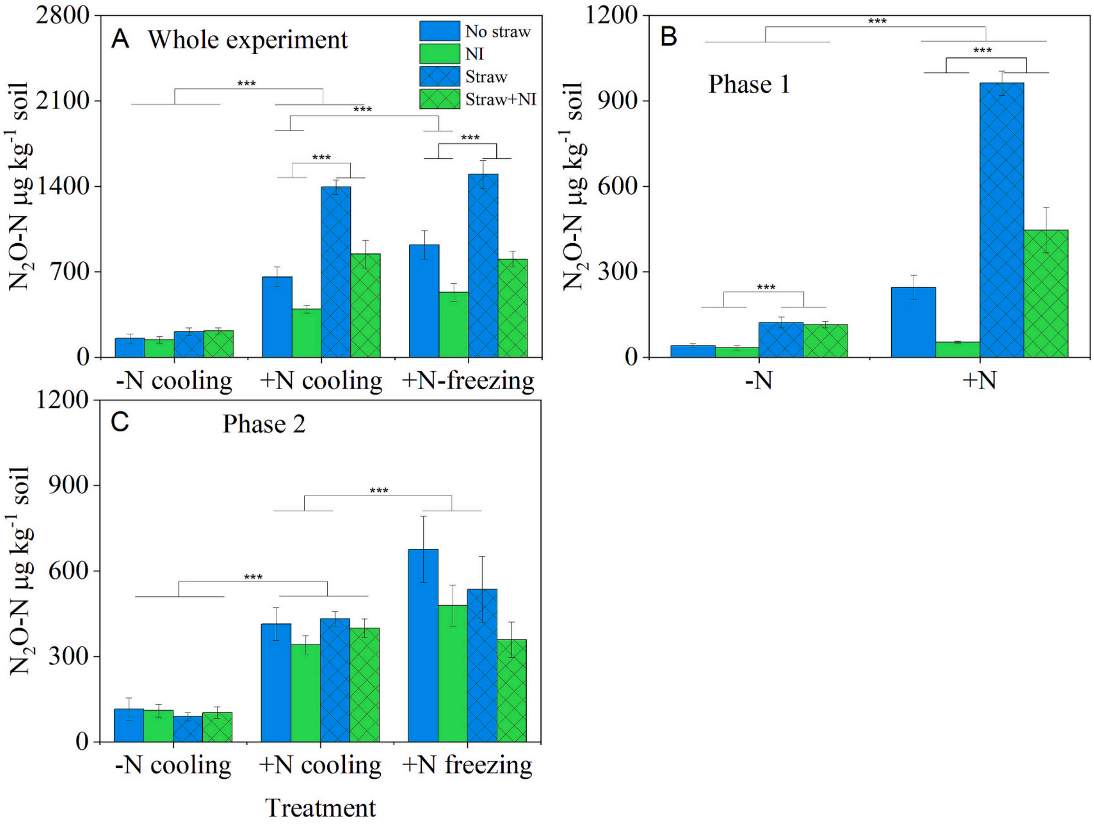

Fig. 4. Cumulative N_2O emission (mean ± 1SE) in treatments without (−N) and with fertilization (+N), without (+N-cooling) and with (+N-freezing) a subsequent freezing-thawing period. Straw incorporation is shown by crosshatched bars and application of nitrification inhibitor (NI) is shown by green colour. A - the whole experiment, B - the Phase 1, and C- the Phase 2 under winter temperatures. Significant treatment and interaction effects as revealed by ANOVA with contrasts is given on the top, with *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

addition (Fig. S1B). Structural equation modeling revealed significant positive associations between N fertilization (0.66) and straw amendment (0.76) with cumulative N_2O emissions, while NI showed a negative association (−0.28) (Fig. S2).

3.2. N remineralization and N_2O emission during the Phase 2 (under winter temperatures)

In unfertilized soils, total N_{min} remained stable throughout the conditioning and temperature fluctuation phases (Fig. 3E–F). In contrast, fertilization doubled (+N) and tripled (+N + NI) N_{min} in the +N cooling treatment without straw after warming (Fig. 3F), mainly due to NO_3^--N accumulation (Fig. 3E). While N fertilization increased soil NO_3^--N 5.5-fold ($p < 0.001$), straw addition reduced it by half ($p < 0.05$). NI led to 46% higher residual NO_3^--N in the fertilized non-straw treatment ($p < 0.001$, Fig. 3F).

NH_4^+-N had increased on average by 80% at the end of Phase 2, with no significant differences between +N-cooling and +N-freezing treatments (Fig. 3B). NI led to strongly enhanced amounts of NH_4^+-N in fertilized treatments ($p < 0.001$), whereas straw addition substantially mitigated this increase ($p = 0.09$).

Fertilization led to fivefold increase N_2O emissions at the end of Phase 2 ($p < 0.001$), while N_2O emissions increased only 2.8-fold in fertilized soils with straw addition ($p < 0.001$; Fig. 4C). Overall, N_2O emissions were 29% higher after freezing than cooling ($p < 0.001$), but not different when both straw and NI were applied ($p = 0.388$; Fig. 4C). NI reduced N_2O losses from fertilized soils by 13 and 31% under cooling and freezing, respectively ($p < 0.001$; Fig. 4C).

3.3. N transformations and functional gene abundances

Denitrifier genes (nirS, nirK, nosZ) were generally more abundant than nitrifier genes. In fertilized soils, freezing significantly boosted gene abundances: nirK, nirS and NosZ increased 3-, 9- and 1.5-fold, respectively, compared to cooling (all $p < 0.001$; Fig. 5). Nitrifier AOB increased 1.5-fold after freezing ($p < 0.001$), but only in fertilized soils with straw addition (Fig. 5D,E).

In −N treatments, straw addition increased nirK, nirS and nosZ gene abundances 3-, 2.6- and 2.7-fold, respectively ($p < 0.01$–0.001), and raised AOA 2.7-fold ($p < 0.001$). N fertilization decreased AOB, nirS and nosZ by 67%, 86.5% and 13.1% ($p < 0.05$), respectively. In +N-cooling treatments without straw, NI increased AOA, AOB, nirK, nirS and nosZ abundances 42.9%, 19.6%, 68.2%, 60.3% and 36.5%, respectively ($p <$ 0.05–0.001, Fig. 5).

3.4. Total N losses caused by leaching and N_2O emissions

Total N losses (gaseous and leached) rose by 50% under N fertilization, but declined with NI and straw addition (Fig. 6). NI reduced N-losses on average by 35.5% across +N-cooling and +N-freezing treatments. The effects of straw depended on fertilization. N-losses decreased by 72.8% in the −N treatment and by 20.6% in the +N treatment. Freezing reduced total N losses by 31.6%, largely due to lower leachate volumes compared with cooling (Fig. S3).

Total Nmin leaching increased by 45% in +N treatments without freezing, while the addition of straw reduced leaching by 69%, on average across all treatments (Fig. S3).

NI had no significant influence except in +N-cooling treatments without straw. Freezing-thawing reduced N leaching by 66.3% compared with cooling.

3.5. Carbon dioxide emissions and global warming potential

Straw amendments increased cumulative CO_2 emissions 8-fold during the Phase 1 ($p < 0.001$; Fig. S4A), and 2-fold during Phase 2 ($p < 0.001$; Fig. S4B). N fertilization and NI had no significant effects.

CO_2 dominated total greenhouse gas (GHG) emissions, while the contribution of methane to the GWP was negligible (Table S1). Straw amendment raised GWP 4.6-fold (Fig. S5). Due to the contribution of N_2O, N fertilization increased GWP by 14.7% under straw addition, whereas NI decreased GWP by 4.6% in fertilized soils.

4. Discussion

4.1. Mitigation of N losses by straw and NI amendment over seasonal temperature fluctuations

Large amounts of N are lost from agricultural soils in winter (Cookson et al., 2002; Henke et al., 2008; Li et al., 2021). We investigated how straw amendment to N-fertilized soils with or without NI application, mitigates N losses in the critical phases of soil conditioning after amendment, and after soil warming following cooling or freezing. Straw addition markedly reduced total N losses (both through leaching and N_2O emissions; Fig. 6) mainly through N immobilization in microbial biomass (Chen et al., 2023; Li et al., 2021; Rosinger et al., 2022b). Despite reducing overall N losses, straw substantially increased N_2O emissions after N-fertilization in Phase 1 (Fig. 4, Fig. 7). However, the reduction in N leaching associated with straw addition offset these additional gaseous losses in all treatments except the +N-freezing treatment without NI. Temperature fluctuations significantly increased total N losses, but losses were lower under +N-freezing than under +N-cooling conditions, due to reduced N leaching after freezing, which outweighed the associated rise in N_2O emissions in N-fertilized soils.

Previous studies have shown that additions of readily decomposable organic C stimulate denitrification by supplying energy to denitrifiers (Burford and Bremner, 1975; Firestone and Davidson, 1989; Weier et al., 1993). However, the effect of straw on soil N_2O emissions is inconsistent, ranging from positive to neutral or even negative, depending on straw quality, climate, and fertilization regime (Wu et al., 2020). In the present study, increased N_2O emissions following combined N fertilization and straw addition (Fig. 4B, Fig. S3) are consistent with conditions that may favour denitrification.

The timing of straw and fertilizer application largely controls straw-induced N_2O emissions. In our study, simultaneous application was associated with increased microbial respiration (Fig.S4), which may have contributed to the formation of transient anoxic microsites during rapid straw decomposition, as suggested in previous studies (Kravchenko et al., 2017), explaining the early N_2O peak in Phase 1 (Fig. 2). In line with the short-lived effect of straw on the N_2O flux, no increase in N_2O emissions was observed in Phase 2 (72 days post-straw application; Fig. 4). By that time, most of the easily accessible added C was decomposed (0.8 of 2.4 mg C added emitted as CO_2; Fig. S4 A), potentially limiting substrate availability for denitrification (Figs. 4 and 6). As expected, NI application significantly reduced total N losses in all N fertilization treatments (Figs. 4, 6, S3). The NI-induced suppression of N_2O emissions was most evident within the four weeks following N application in Phase 1 (Fig. 4, Table S1), when most NH_4^+-N had been converted to NO_3^--N or immobilized in microbial biomass under straw amendment (Figs. 3A, C; and Chen et al., 2023 Fig. 2D). In contrast to the strong reduction in N_2O emissions (Fig. 4).

The main inhibitory effect of NI on nitrification was short-lived, persisting only during the four-week conditioning phase (Phase 1). After this period, nearly all applied NH_4^+-N fertilizer was oxidized to NO_3^--N (Fig. 3A, C), leaving only about one-tenth of the added NH_4^+-N at day 28. Despite this transient suppression, NI significantly reduced the first N_2O emission peak (Fig. 2), likely due to the temporary shortage of NO_3^--N as electron acceptor for denitrification during this early 'hot moment' (Groffman et al., 2009). Although NI effects on N_2O emissions remained significant during Phase 2, the effects of NI in fertilized treatments were threefold lower as compared to Phase 1 (Fig. 4B and C).

During Phase 2, NI increased soil NH_4^+-N contents in treatments without straw, probably due to suppression of nitrification of remineralized fertilizer N (significant NI × Straw interaction; Fig. 3B). Straw addition masked this effect potentially due to promoting microbial N immobilization, as previously observed in a urea-straw- NI combination experiment (Ma et al., 2019).

A complementary interaction between straw and NI was evident: straw reduced N leaching through microbial immobilization (Chen et al., 2023), while NI decreased N_2O emissions, effectively counteracting gaseous N losses. However, these interactions should be interpreted cautiously, as the underlying mechanisms were not directly quantified. Since leaching accounted for only about 1% of fertilizer N in this experiment, under field conditions, straw likely mitigates N losses much more effectively, as leaching in our experiment was substantially lower than typical field values reported over prolonged precipitation (Abdalla et al., 2019). While these findings provide mechanistic insights, it should be noted that mesocosm conditions may not fully capture field-scale variability in soil structure and hydrological processes.

4.2. Effect of cooling vs. freezing on N_2O emissions and N losses

The second hypothesis, stating that soil N losses are driven by freezing-thawing rather than seasonal cooling was supported for N_2O emissions, but not for total N losses. Freezing increased N_2O emissions compared to cooling (Fig. 4C), though the difference was modest (5.5–37.9%) because treatment effects were compared to cooling rather than to a stable-temperature control, as in most previous studies (Song et al., 2017; Wagner-Riddle et al., 2017). Notably, already the cooling-warming sequence in Phase 2 triggered a strong N_2O emission peak, despite initially low emissions in Phase 1 (Fig. 2).

In contrast, total N losses (N_2O plus leaching) were higher after cooling than freezing in treatments without straw (Fig. 6). During the subsequent warming phase, N-fertilized mesocosms showed a N_2O peak and rise in soil NH_4^+-N and NO_3^--N, reflecting residual effects of the applied fertilizers (Figs. 3B, D, 4C). Mineral N levels at the end of Phase 2 exceeded those after Phase 1, suggesting remineralization and nitrification of previously immobilized fertilizer N (Fig. 3B and D). Similar temperature-driven increases in soil NO_3^- and turnover rates have been observed by Cookson et al. (2002).

These results highlight the potential for substantial N losses may occur not only following freeze-thaw events but also during mild soil cooling–warming phases typical of late winter or early spring (Cameron et al., 2013). To mimic field conditions, soil water content was raised during leachate collection (day 62), possibly promoting denitrification and contributing to the observed N_2O peak (Schlüter et al., 2025; Zhang et al., 2026). However, this interpretation should be treated with care because relatively short experimental duration cannot fully represent soil N dynamics under field conditions. Although leachate was collected two months after fertilization, when most NH_4^+-N had already been converted to NO_3^-, the initial NI effect was still visible from N leaching rates in fertilized treatments without straw following freezing-thawing.

4.3. Interplay of nitrification and denitrification and N losses from soil

During the first two weeks of the experiment, when the initial emission peak occurred, both nitrification and denitrification likely contributed to N_2O production, although microbial functional gene abundances were only assessed at the end of the experiment and therefore cannot be directly linked to these earlier emission dynamics. By the end of this phase, nitrate dominated the mineral N pool (Fig. 3), which is consistent with substantial nitrification across all treatments, even including NI treatments. Ongoing nitrification under varying temperatures was associated with higher NO_3^- accumulation in both +N-cooling and +N-freezing treatments compared to Phase 1 (cf. Fig. 3C and D). Archaean AOA showed greater resilience to +N-cooling and +N-freezing treatments than bacterial AOB (Fig. 5D and E). Similar patterns were observed in previous studies where AOA maintained activity after periodic freezing (Tzanakakis et al., 2020), suggesting that archaea may play an important role in nitrification shortly after soil freezing. However, it should be noted that gene abundance does not directly reflect microbial activity or process rates.

Straw addition was associated with denitrifier gene abundance (*nirK*, *nirS* and *nosZ* genes) only in unfertilized (−N) treatments during Phase 2, likely because temperature and pH effects masked the effect of straw addition in fertilized soils. This highlights the temporal variability in gene abundance reported previously (Wertz et al., 2013; Zhang et al., 2023). Compared to the cooling-warming treatment, freezing-thawing increased the abundance of *nirK, nirS* and *nosZ* genes (Fig. 5 A–C). The relatively smaller increase in *nos*Z compared to *nir*K may be consistent with a reduced potential for N_2O reduction, which could contribute to higher N_2O emissions (Sennett et al., 2024). These interpretations are based on gene abundance data and should not be taken as direct evidence of process rates. Since the soil was sampled two weeks after thawing and the subsequent N_2O peak (Figs. 1 and 2), the delay allowed a clearer detection of the positive response of denitrifiers to freezing, compared to earlier studies that used shorter sampling intervals (Wertz et al., 2013, 2016; Kazmi et al., 2023). However, the lack of temporally resolved microbial data limits our ability to establish direct relationships between microbial community dynamics and N fluxes over time.

A marked difference between *nir*S and *nir*K gene abundance (+N-cooling) was linked to a 0.5 pH unit decrease in fertilized mesocosms (Chen et al., 2023). As *nir*S nitrifiers are less tolerant to soil acidity, they could have been outcompeted by *nir*K-type microorganisms (Bowen et al., 2020). This pH-related effect was absent after freezing and thawing (+N-freezing), suggesting that other environmental factors may have influenced the pattern under these conditions.

5. Conclusions

In arable soils, high residual N levels after harvest can lead to substantial N losses, particularly in the absence of post-harvest vegetation such as cover crops. Our results show that straw residue incorporation combined with nitrification inhibitors can effectively mitigate these losses. Straw incorporation stimulated microbial biomass growth and temporarily increased microbial N immobilization, while nitrification inhibitors suppressed the microbial oxidation of ammonium to nitrate, thereby enhancing ammonium retention and reducing the potential for denitrification.

Accordingly, straw incorporation markedly reduced N leaching, whereas nitrification inhibitors substantially decreased N_2O emissions. The combination of both measures proved particularly effective in mitigating post-harvest N losses, and these effects were consistent under both cooling-warming and freezing-thawing temperature regimes.

Importantly, our results demonstrate that not only freeze–thaw events but also moderate soil cooling followed by warming can substantially increase greenhouse gas emission potential. This finding highlights that significant N losses may occur even during relatively mild winter temperature fluctuations typical of late winter or early spring. Moreover, soil freezing strongly enhanced N_2O emissions in treatments without straw incorporation or nitrification inhibitor application, indicating that soil freezing can trigger considerable N losses from agricultural soils when appropriate mitigation measures are not implemented. However, the extent to which these findings apply to field conditions remains uncertain. Under climate change, declining snow cover is expected to reduce soil insulation and increase the frequency and intensity of soil freezing events, thus more research on mitigation measures is urgently required in field systems, where soil structure, hydrology, plant interactions, and long-term dynamics may differ substantially.

Taken together, the combined use of straw incorporation and nitrification inhibitors represents a promising strategy to mitigate post-

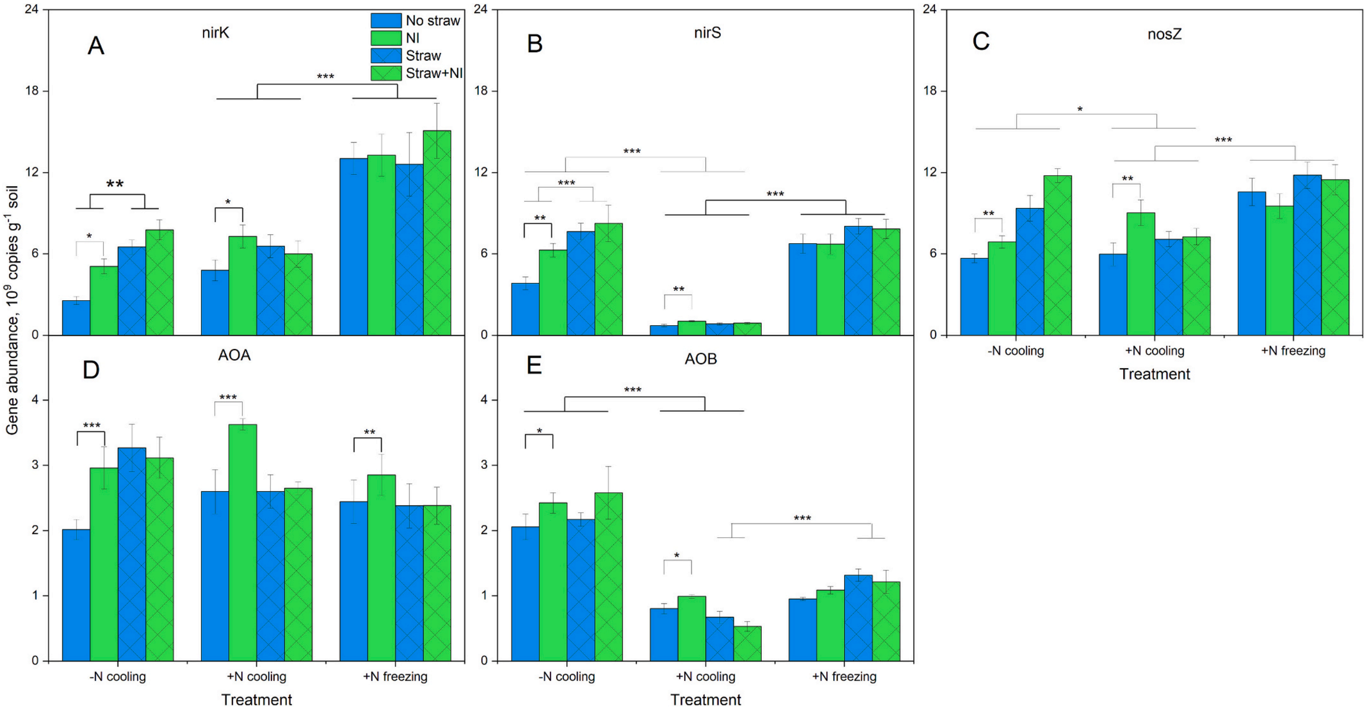

Fig. 5. N cycling functional gene abundances of denitrifiers: NO$_2^-$ reductase nirK (A) and nirS (B), nitrous oxide reductase nosZ (C), and ammonia-oxidizing bacteria (AOB - D) and archaea (AOA - E) under different treatments measured at the end of the experiment. The treatments +N and −N refer to the unfertilized and fertilized mesocosms, respectively; +N-cooling and +N-freezing refer to the fertilized mesocosms without and with freezing-thawing, respectively. Straw incorporation is shown by crosshatched bars and application of nitrification inhibitor (NI) is shown by green colour. Given is the mean ± 1SE (n = 7). Significant treatment and interaction effects as revealed by ANOVA with contrast is given on the top, with *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

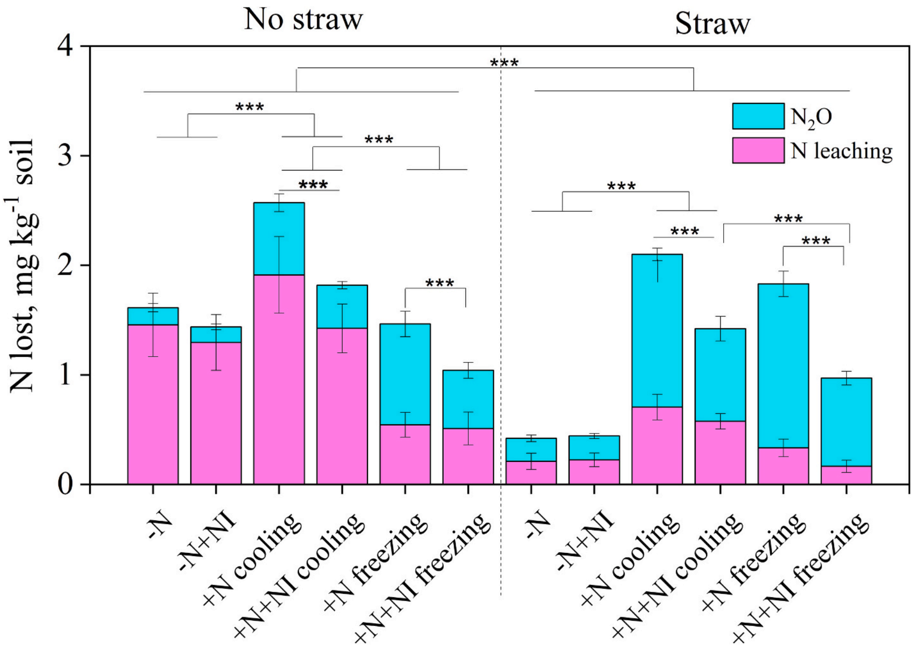

Fig. 6. N lost from soil as N_2O and NO_3^- in leachate under different treatments during the whole experiment. The treatments −N and +N refer to the unfertilized and with N fertilizer, respectively; NI refers to the treatments with nitrification inhibitor application. Given is the mean ± 1SE (n = 4). Significant treatment and interaction effects as revealed by ANOVA with contrast is given on the top, with *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

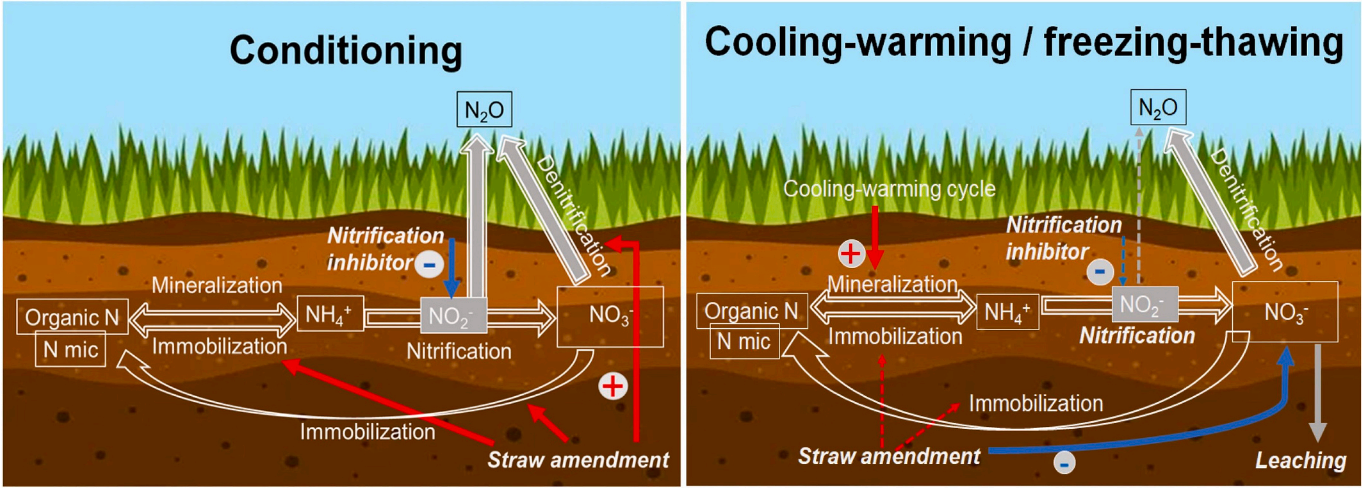

Fig. 7. Soil N cycle and N_2O emission pathways before and after seasonal temperature change: cooling-warming and freezing-thawing cycles. The thick solid arrows present strong effects, while the thin dashed arrows present weak effects. The stimulating effects are shown by the red arrows, and the inhibiting effect by the blue arrows, the gray-shaded arrows show the N losses from the system.

harvest N losses from arable soils. By enhancing soil N retention and reducing gaseous and leaching losses, this management approach has the potential to improve N availability for subsequent crops while lowering the greenhouse gas footprint of agricultural systems.

CRediT authorship contribution statement

Hao Chen: Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Sergey Blagodatsky:** Writing – review & editing, Validation, Supervision, Methodology, Data curation. **Christoph Rosinger:** Writing – review & editing, Supervision, Project administration, Methodology, Data curation, Conceptualization. **Rüdiger Reichel:** Writing – review & editing, Validation, Methodology, Data curation. **Bo Li:** Validation, Software, Data curation. **Amit Kumar:** Writing – review & editing, Methodology, Data curation. **Steffen Rothardt:** Methodology, Formal analysis, Data curation. **Nicolas Brüggemann:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Henning Kage:** Writing – review & editing, Resources, Methodology, Conceptualization. **Michael Bonkowski:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2026.107083.

Data availability

Data will be made available on request.

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