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High soil moisture rather than drying-rewetting cycles reduces the effectiveness of nitrification inhibitors in mitigating N_2O emissions

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Abstract

Climate change has been intensifying soil drying and rewetting cycles, which can alter the soil microbiome structure and activity. Here we hypothesized that a soil drying-rewetting cycle enhances biodegradation and, hence, decreases the effectiveness of nitrification inhibitors (NIs). The effectiveness of DMPP (3,4-Dimethylpyrazole phosphate) and MP + TZ (3-Methylpyrazol and Triazol) was evaluated in 60-day incubation studies under a drying and rewetting cycle relative to constant low and high soil moisture conditions (40% and 80% water-holding capacity, WHC, respectively) in two different textured soils. The measurements included (i) daily and cumulative N_2O-N emissions, (ii) soil NH_4^+-N and NO_3^--N concentrations, and (iii) the composition of bacterial soil communities. Application of DMPP and MP + TZ reduced the overall N_2O-N emissions under drying-rewetting (-45%), as well as under 40% WHC (-39%) and 80% WHC (-25%). DMPP retarded nitrification and decreased N_2O-N release from the sandy and silt loam soils, while MP + TZ mitigated N_2O-N production only from the silt loam soil. Unexpectedly, between days 30 and 60, N_2O-N emissions from NI-treated soils increased by up to fivefold relative to the No-NI treatment in the silt loam soil at 80% WHC. Likewise, the relative abundance of the studied nitrifying bacteria indicated that the NIs had only short-term effectiveness in the silt loam soil. These results suggested that DMPP and MP + TZ might trigger high N_2O-N release from fine-textured soil with constant high moisture after this short-term inhibitory effect. In conclusion, DMPP and MP + TZ effectively reduce N_2O-N emissions under soil drying and rewetting.

Keywords DMPP · Ammonia-oxidizing bacteria · Denitrification · Climate change · Drought · Nitrous oxide

Introduction

Climate change has been causing more frequent extreme weather events, such as droughts and heavy precipitation (IPCC 2021). Consequently, soil drying and rewetting cycles have been intensified globally. These cycles can tremendously impact the emission of greenhouse gases (GHG) from agricultural lands by altering soil microbiome structure and activity (Friedl et al. 2022; Jiao et al. 2023). Soil drying may cause the breakdown of aggregates and expose protected organic C (SOC). Similarly, the release of accumulated soluble substances due to microbial cell rupture after sudden rewetting may stimulate chemical reactions and microbial activity (Liu et al. 2018). Consequently, soil rewetting might increase N_2O emissions due to accumulated substrates for denitrification and enhanced microbial activity (Pezzolla et al. 2019).

The application of nitrification inhibitors (NIs) has been suggested to control N_2O release from agricultural soils (Ribeiro et al. 2023). NIs can delay the oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) by inhibiting the activity of ammonia-oxidizing bacteria (AOB) (Hayden et al. 2021; Ruser and Schulz 2015). In a meta-analysis considering field experiments, Yang et al. (2016) showed that the NI 3,4-Dimethylpyrazole phosphate (DMPP) efficiently delayed NH_4^+ conversion to nitrate (NO_3^-), thereby reducing its leaching and mitigating N_2O release by 48%. Similarly, DMPP has been

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suggested to be an efficient NI under different moisture and temperature conditions (Guo et al. 2022). In addition, the NI consisting of a mixture of 3-Methylpyrazol and Triazol (MP + TZ) has been utilized in Germany and found to reduce N_2O emissions (Fan et al. 2022; Guo et al. 2021; Scheer et al. 2017; Wang et al. 2020; Wu et al. 2017). Wang et al. (2020) reported that MP + TZ decreased N_2O emissions by 85% and 52% from urea and urea ammonium nitrate application, respectively. Despite some studies indicating that MP + TZ can delay nitrification and mitigate N_2O production (Fan et al. 2022; Pietzner et al. 2017), the impacts of this inhibitor on soil microbial communities were not investigated.

The efficiency of NIs relies on numerous factors such as temperature, moisture, pH, texture, SOC, tillage, NI, and fertilizer types and rates (Ribeiro et al. 2023). For instance, Barth et al. (2019) indicated that DMPP is less effective in clayey soils due to adsorption on soil particles and enhanced microbial activity. Nauer et al. (2018) showed that DMPP was ineffective in reducing N_2O emissions from urea. Mazzetto et al. (2015) and Barneze et al. (2015) reported that N_2O release from cattle urine patches in grasslands might not be reduced by NIs, probably due to warm temperatures. In addition, changes in soil moisture content are an important factor influencing NI effectiveness (Barrena et al. 2017; Di et al. 2014; Guo et al. 2022; Menéndez et al. 2012; Zhang et al. 2023). Likewise, NIs could become ineffective under soil drying and rewetting cycles due to enhanced microbial activity, which might accelerate their biodegradation. However, to the best of our knowledge, studies investigating the effect of drying and rewetting on the effectiveness of NIs are lacking.

Therefore, we hypothesized that (i) a soil drying-rewetting cycle enhances the biodegradation of NIs and weakens their nitrification inhibition effect and (ii) DMPP has a more stable effect on delaying nitrification over different soil textures and moisture conditions than MP + TZ. This study aimed to evaluate the effectiveness of DMPP and MP + TZ under a drying and rewetting cycle relative to constant low and high soil moisture conditions in coarse- and fine-textured soil. The performance of NIs was measured in terms of (i) daily and cumulative N_2O-N emissions, (ii) soil NH_4^+-N and NO_3^--N concentrations, and (iii) the composition of bacterial soil communities over the experimental period. To our knowledge, this is the first study to investigate the impacts of soil drying and rewetting on NI effectiveness.

Material and methods

Site and soil description

Sandy and silt loam soils were collected from agricultural sites in Schleswig–Holstein, northern Germany (54°00′42.9"

N 8°55′43.9" E and 54°12′24" N 10°0′59" E, respectively). Subsequently, the soils were air-dried, passed through a 2 mm sieve, and homogenized. The main properties of the sandy soil were: sand, 934 g kg^{-1}; silt, 50 g kg^{-1}; clay, 16 g kg^{-1}; SOC, 8.3 g kg^{-1}; total N, 0.8 g kg^{-1}; NH_4^+-N, not detected; NO_3^--N, 2.93 mg kg^{-1}; bulk density, 1.4 g cm^{-3}; pH$_{CaCl2}$, 6.2; water-holding capacity (WHC), 0.27 g g^{-1}. The main properties of the silt loam soil were: sand, 160 g kg^{-1}; silt, 620 g kg^{-1}; clay, 220 g kg^{-1}; SOC, 10 g kg^{-1}; total N, 1.4 g kg^{-1}; NH_4^+-N, 1.53; NO_3^--N, 50.91 mg kg^{-1}; bulk density, 1.3 g cm^{-3}; pH$_{CaCl2}$, 7.2; WHC, 0.35 g g^{-1}.

Incubation experiments

Two incubation experiments were carried out at the Institute of Plant Nutrition and Soil Science, Kiel University, Germany. These incubation experiments were conducted in a climatic chamber with 25 °C temperature and 45% relative humidity. The objective of carrying out this study under completely controlled environmental conditions was to isolate the influence of NI type and soil water content from other influencing factors. We utilized a completely randomized design with four replicates. The investigated factors were NI type (MP + TZ, DMPP, and No-NI) and soil water content (40% WHC, 80% WHC, and drying-rewetting). We utilized 40% and 80% WHC as the minimum and maximum soil moisture levels because the existing literature on NI research has adopted these as references to investigate NI performance when N_2O-N emissions are expected to originate mainly from nitrification and denitrification processes, respectively (Guo et al. 2022; Torralbo et al. 2017; Zhang et al. 2023). Therefore, the same thresholds were utilized to allow the comparison of our study with the current literature. Furthermore, treatment with steady soil moisture at 60% WHC, which is the average moisture of the simulated drying-rewetting cycle, was not added to the experimental design because previous research already showed that the NIs utilized in our study are efficient at 60% WHC steady soil moisture (Guo et al. 2022; Menéndez et al. 2012; Zhang et al. 2023).

Thirty-six cylindrical pots (15 cm diameter and 33 cm length) were filled with soil up to 20 cm high. Ammonium sulfate ($(NH_4)_2SO_4$) was used as N fertilizer at a 0.25 g NH_4^+-N kg^{-1} soil application rate. Both DMPP and MP + TZ were applied at 1% of the applied NH_4^+-N. MP + TZ was being commercialized under the name PIADIN®, which represents the combination of the active ingredients 3-Methylpyrazol (1.5%) and Triazol (3%) in urea ammonium nitrate solution. The concentration of ammonium nitrate in PIADIN® was 38% (mass), and the remaining constituents were not specified by the manufacturer (Kösler et al. 2019). Considering DMPP, the application rate of 1% of the added NH_4^+-N was utilized because it is within the range allowed

by the European Union regulations and in agreement with the concentration used in commercial fertilizers containing DMPP (European Commission 2014). The same concentration was utilized for MP + TZ because it is within the range of application rates recommended by the manufacturer, e.g., 7 L ha^{-1} of the commercial product PIADIN®. The No-NI treatment received only ammonium sulfate without NI application. N fertilizer and NIs were dissolved in deionized water and surface applied on the soil surface through a 100 mL solution at the first day of incubation.

Considering the 40% and 80% WHC treatments, the soil water content was monitored daily and readjusted to the respective values by adding deionized water. For the drying-rewetting treatment, the soil water content was maintained initially at 80% of WHC at the start of the experiment (day 0). When the soil moisture content reached 40% of WHC, it was re-adjusted to 80% of WHC to simulate a drying-rewetting cycle, as demonstrated in Fig. 1.

Sampling, measurement, and calculation of N$_2$O-N emissions

For N$_2$O-N sampling, air-tight lids were placed on the pots, and the gas samples were collected with 10 mL syringes at intervals of 0, 20, 40, and 60 min. Subsequently, the samples were stored in pre-evacuated exetainers. Except for the sampling period, the pots were kept open. Gas samples were collected periodically from the headspace of the pots to measure the N$_2$O concentration by gas chromatography (Agilent 7890A GC, Agilent). Linear interpolation was utilized to

Fig. 1 Representation of the drying-rewetting cycle implemented in the incubation experiments. Asterisks represent the sampling days for mineral N measurements. Arrows indicate the sampling days for microbiological analysis. WHC = water-holding capacity

estimate N$_2$O-N emission for the days gas sampling was not performed. Daily emissions were divided into Phase 1 (0 to 30 days of incubation) and Phase 2 (31 to 60 days of incubation) because of the different patterns of N$_2$O-N emissions in each phase and to ease data analysis, discussion, and interpretation. Finally, the cumulative N$_2$O-N emission was calculated from the daily emissions.

Sampling and analysis of soil NH$_4^+$ and NO$_3^-$

Soil samples were collected after 15, 30, 45, and 60 days of incubation from the 0–20 cm depth for NH$_4^+$ and NO$_3^-$ measurements. Considering the drying and rewetting treatment, soil samples were taken prior to soil rewetting on the 30th day of incubation. The soil samples were extracted with 1 M KCl solution (1:10 ratio) by shaking for 1 h in a shaker with a vertical rotation speed of 60 rpm. Subsequently, the suspensions were filtered, and the filtered extracts were analyzed for NH$_4^+$ and NO$_3^-$ concentrations using a continuous flow analyzer (San^{++} Continuous Flow Analyzer, Skalar). Additionally, a sub-sample of soil was oven-dried at 105 °C for 24 h to adjust for the water content.

Soil DNA extraction, 16S rRNA gene amplicon sequencing and analysis

Samples of silt loam soil collected on the 15th and 45th day after incubation were selected for microbial community analysis, and 0.5 g of soil was used for the genomics DNA extraction using Fast DNA SPIN Kit for Soil (MP Biomedicals). Only this silt loam soil was utilized for microbial analysis to investigate the increased N$_2$O-N emissions from NI-treated soil in Phase 2. The silt loam soil was selected because fine-textured soils present greater microbial abundance than coarse-textured ones (Guo et al. 2021).

For bacterial sequencing library preparation (in three technical replicates), primer pair targeting 16S rRNA gene V3-V4 region (341F 5'-CCTACGGGAGGCAGCAG-3' and 806R 5'-GGACTACHVGGGTWTCTAAT-3') was used for 16S rRNA gene amplicon sequencing. Sequencing library preparation and MiSeq paired-end (2 × 300 bp) sequencing (Illumina, v3 chemistry) on the sample pool was performed at the Center of Molecular Life Sciences (ZMB), Kiel University. ZymoBIOMICSTM Microbial Community DNA Standard was used for sequencing, data analysis, and quality control for the amplicon sequencing. 16S rRNA gene amplicon sequencing data were analyzed on the de-multiplexed fastq reads. Cutadapt (v3.5) (Martin 2011) was used for the adapter and primer removal and quality control (Q-score > 20). On average, 46,440 de-multiplexed fastq paired-end reads per sample were obtained, and 45,829 (94%) were retained after adapter trimming and quality filtering. The quality controlled paired end reads were merged
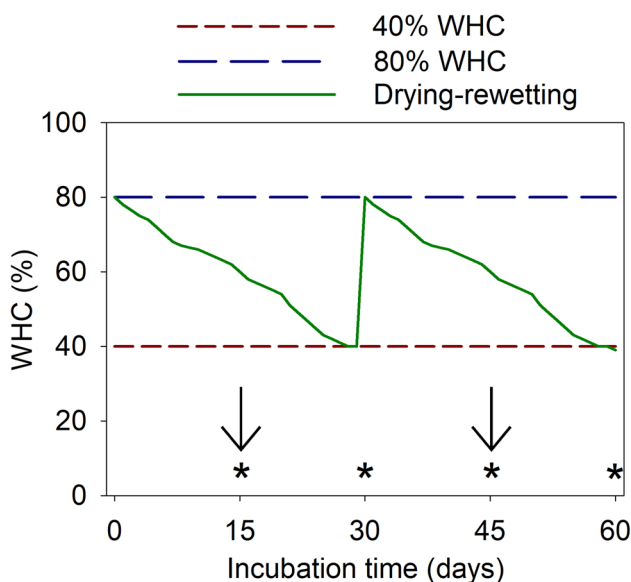
using VSEARCH (v2.21.1) (Rognes et al. 2016), and 22,434 merged reads (merging percentage = 96.5%) were obtained. Chimeric sequence removal and generation of amplicon sequence variants (ASV) were performed on the merged sequences using package *dada2* (v1.22.0) (Callahan et al. 2016) in RStudio (v2021.09.0+351) (RStudio Team 2020) running R (v4.1.3) (R Core Team 2021).

On average, 17,132 reads per sample were non-chimeric and used to analyze sequence variants, resulting in 8,295 ASVs. Taxonomic annotations of ASVs were performed using the 16S rRNA database formatted for DADA2 with Genome Taxonomy Database taxonomies (v202) (Alishum 2021). The abundance table, taxonomy table, sample metadata, and phylogenetic tree were merged into a single object and used for visualization and statistical analysis using packages *phyloseq* (v1.38.0) (McMurdie and Holmes 2013), *vegan* (v2.6.2) (Oksanen et al. 2019), *ggplot2* (v3.3.6) (Wickham 2016). Out of 8,295 ASVs, ten, 8,284, and one ASV were taxonomically classified (at kingdom level) to Archaea, Bacteria, and Unknown taxa, respectively (Table 1)

Only bacterial ASVs (8,284) were considered for further analysis, and the remaining ASVs were discarded. The differential abundance testing and correlation analysis for the genera related to nitrifying bacteria was done and visualized using package *DESeq2* (v1.34.0) (Love et al. 2014), stats (v4.2.2), *pheatmap* (1.0.12) (Kolde 2012) and ggcor (0.9.4.3) (Huang et al. 2020). MiSeq sequencing de-multiplexed paired-end fastq reads were deposited in SRA at NCBI under accession number PRJNA1018126.

Statistical analysis of N_2O-N cumulative emissions

The data for cumulative N_2O-N emissions were subjected to the normality test. Subsequently, a two-way analysis of variance (F test) was performed for the factors NI and soil moisture within each experimental phase and soil texture. The response variables that presented significant treatment effects were submitted to the Tukey test ($p < 0.05$) using the R software (R Core Team 2021).

Results

N_2O-N fluxes and cumulative emissions

The overall N_2O-N emission from soils was higher at 80% WHC than at drying-rewetting and at 40% WHC (Table 1, Fig. 2). Furthermore, DMPP and MP + TZ reduced the overall N_2O-N release relative to No-NI by 43% and 20%, respectively (Table S1).

In the sandy soil, MP + TZ significantly reduced N_2O-N emission only under drying-rewetting in Phase 1. However, this inhibitor mitigated N_2O-N release under all tested moisture levels in the silt loam soil in Phase 1. DMPP significantly decreased N_2O-N emissions in almost all investigated combinations of soil textures and moisture levels (Table 2). On average, applying NIs reduced N_2O-N emissions by 45% under drying-rewetting, 39% under 40% WHC, and 25% under 80% WHC (Table S1). Furthermore,

Table 1 Cumulative N_2O-N emissions (Mean ± SD) from untreated (No-NI) and MP + TZ or DMPP treated sandy and silt loam soils fertilized with ammonium sulfate and subject to different moisture conditions

	Sand soil				Silt loam soil			
	Emission (µg kg^{-1})	Change (%)	Emission (µg kg^{-1})	Change (%)	Emission (µg kg^{-1})	Change (%)	Emission (µg kg^{-1})	Change (%)
	Phase 1		Phase 2		Phase 1		Phase 2	
40% WHC								
No-NI	6.7 ± 1.6 ns		23.2 ± 2.6a		30.5 ± 11.6a		10 ± 0.7a	
MP + TZ	6.6 ± 0.9	-2	20.9 ± 1.3a	-10	14.9 ± 1.4b	-51	11.1 ± 4.4a	+11
DMPP	5.6 ± 1.9	-16	9.9 ± 1.9b	-57	11.6 ± 2.3b	-62	5.8 ± 1.3b	-42
80% WHC								
No-NI	396.8 ± 62.4a		796.5 ± 56.2a		386.1 ± 76.8a		41.7 ± 11.1b	
MP + TZ	323.3 ± 29.1a	-19	773.3 ± 107.2a	-3	145.2 ± 40.9b	-62	190.4 ± 39.1a	+356
DMPP	172.1 ± 20.3b	-57	467.6 ± 44b	-41	114.3 ± 31.7b	-70	261.2 ± 80.9a	+526
Drying-rewetting								
No-NI	138.1 ± 22.4a		338.2 ± 55a		185.9 ± 64.9a		100.7 ± 12.7a	
MP + TZ	32.5 ± 6.6b	-76	330.9 ± 45.5a	-2	63.5 ± 13.9b	-66	60.2 ± 18.2b	-40
DMPP	20.6 ± 4.3c	-85	236.9 ± 30.1b	-30	42.6 ± 14.3b	-77	52.3 ± 18.5b	-48

Change (%) indicates the increase or decrease caused by MP + TZ or DMPP treatments relative to the respective No-NI treatment. WHC = water-holding capacity. Values followed by the same letters in a column are not significantly different according to the Tukey test ($p \leq 0.05$). ns: not significant

on average, DMPP and MP + TZ reduced N_2O-N release by 58% in Phase 1 and 8% in Phase 2. The N_2O-N emission from DMPP and MP + TZ treatments peaked during Phase 2 in the silt loam soil, resulting in an up to fivefold increase relative to the No-NI treatment.
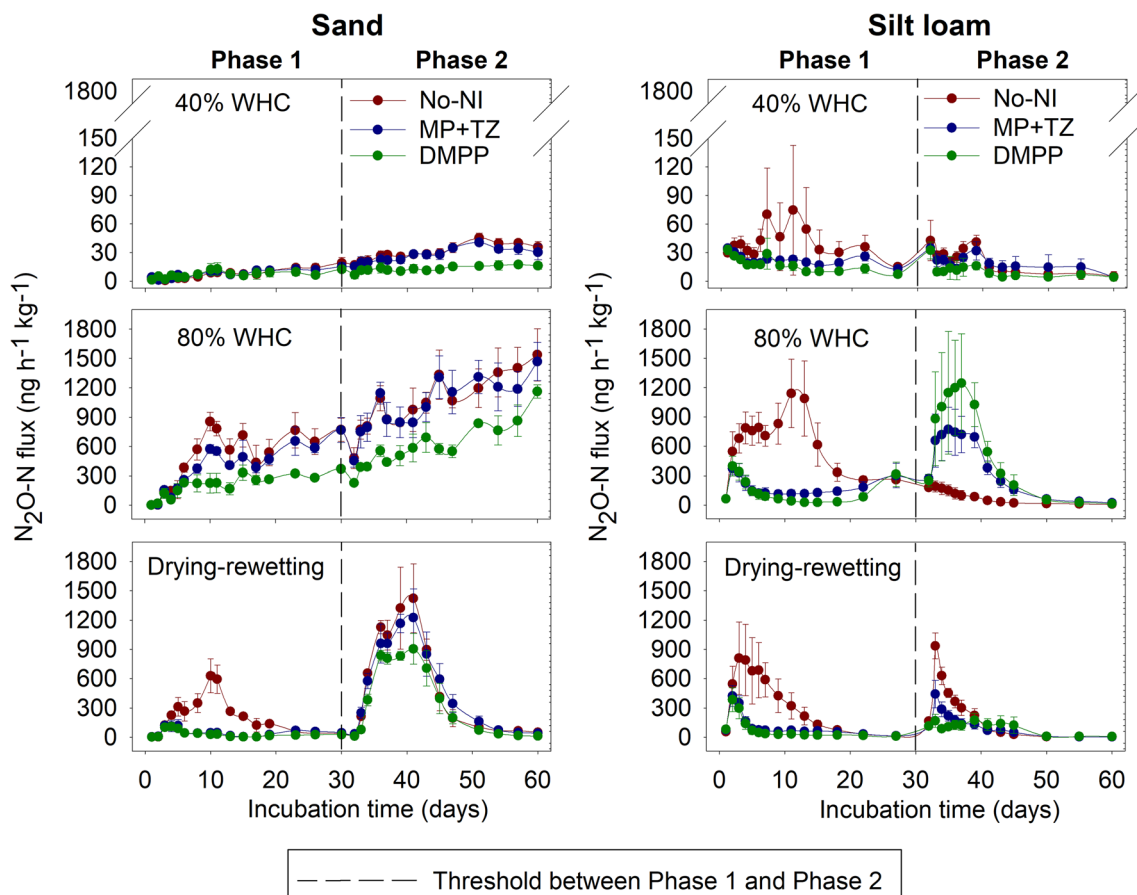
Soil NH_4^+-N and NO_3^--N concentrations

The nitrification delaying effect of DMPP and MP + TZ was more evident in the silt loam soil than in the sandy soil (Fig. 3). Both inhibitors delayed nitrification within the silt loam soil under all soil moisture conditions. For instance, under 40% WHC, NO_3^--N reached higher concentrations than NH_4^+-N at 45 days after incubation in No-NI, while DMPP and MP + TZ resulted in similar NH_4^+-N and NO_3^--N concentrations. For the drying-rewetting and 80% WHC treatments, the concentration of NO_3^--N was greater than that of NH_4^+-N at 30 days after incubation in the No-NI treatments, while the application of DMPP and MP + TZ kept analogous levels of NO_3^--N and NH_4^+-N.

Composition of bacterial soil communities

Application of DMPP and MP + TZ did not affect the number of reads classified as bacteria in amplicon libraries, which ranged between 15,000 and 20,000 reads for all treatments, and the Shannon index (Figures S1 and S2). However, the NIs reduced the relative abundance of the nitrifying bacteria (Fig. 4). Considering the 40% WHC, GW-*Nitrospira*-1 and *Nitrosomonas* presented higher relative abundance in No-NI than in DMPP and MP + TZ. Under drying-rewetting, the relative abundance of *Nitrospira_C*, *Nitrospira*_A, *Nitrosospira*, *Nitrosomonas,* and *Nitrobacter* was decreased by DMPP and MP + TZ. Additionally, *Nitrosococcus*, *Nitrosospira,* and *Nitrobacter* were reduced by NI application under 80% WHC.

The relative abundance of *Nitrosospira* and *Nitrobacter* was positively correlated with N_2O-N emissions (Fig. 6). Soil NH_4^+-N concentration showed a negative correlation with *Nitrospira_C* and A, whereas it had a positive correlation with *Nitrosococcus* and *Nitrobacter*. Soil NO_3^--N concentration was positively correlated with *Nitrospira* C and negatively correlated with *Nitrobacter* and *Nitrosococcus*.

Fig. 2 Daily N_2O-N emissions from untreated (No-NI) and MP + TZ or DMPP treated sandy and silt loam soils fertilized with ammonium sulfate and subject to different moisture conditions
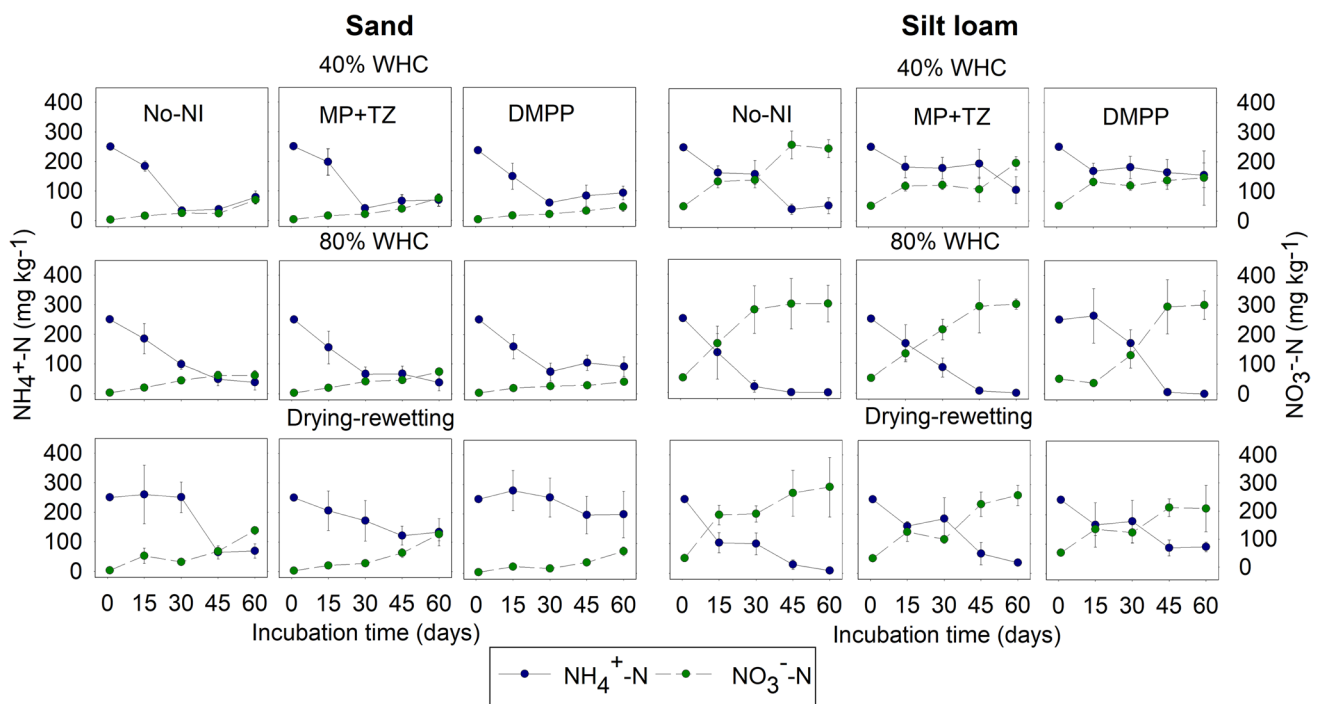
Fig. 3 NH_4^+-N and NO_3^--N concentrations in untreated (No-NI) and MP + TZ or DMPP treated sandy and silt loam soils fertilized with ammonium sulfate and subject to different moisture conditions

In general, the relative abundance of the studied nitrifying bacteria was greater at day 15 than at day 45 in the No-NI treatment for all moisture levels. The opposite trend was shown for DMPP and MP + TZ in which the relative abundance of bacterial genera such as *Nitrobacter* and *Nitrosococcus* increased at day 45 relative to day 15 (Fig. 6).

Discussion

Soil drying and rewetting events are becoming more common due to climate change, and may decrease the effectiveness of NIs due to the enhanced microbial activity upon rewetting. The present study is the first to evaluate the performance of NIs under soil drying and rewetting. Thus, the effectiveness of DMPP and MP + TZ was evaluated under a drying and rewetting cycle relative to constant low and high soil moisture conditions in coarse- and fine-textured soils.

Short-term effect of DMPP and MP + TZ on nitrification and mitigation of N_2O-N emissions

According to the soil mineral N concentrations, it was found that DMPP and MP + TZ delayed the nitrification process under all tested conditions (Fig. 3). In our study, autotrophic nitrification was assumed to be the main process driving the soil NO_3^--N accumulation and N_2O-N release because of the

high NH_4^+-N application as ammonium sulfate. In addition, it has been suggested that NH_4^+-N fertilization mitigates the utilization of organic N by soil microbes (Parajuli et al. 2022). Therefore, it is improbable that heterotrophic nitrification contributed to NO_3^--N and N_2O-N production in this study despite its importance has been proven under different agroecosystems and soil conditions (Gao et al. 2023).

Unlike the silt loam soil, NO_3^--N gradually increased while NH_4^+-N sharply declined in the sandy soil (Fig. 3). The NH_4^+-N fertilizer was applied to the soil by dissolving it in a 100 mL solution to avoid ammonia (NH_3) volatilization. However, such results indicated that other N loss paths, such as NH_3 volatilization, might have occurred probably because of the near-neutral pH and low cation exchangeable capacity of the sandy soil.

In the silt loam soil, DMPP and MP + TZ retarded nitrification for up to 30 days. However, after 30 days of incubation in the silt loam soil, the NO_3^--N exceeded NH_4^+-N concentration in DMPP and MP + TZ treated soils under 80% WHC and drying-rewetting. Similarly, at 60 days after incubation, NO_3^--N and NH_4^+-N concentrations were similar under NI-treated soils at 40% WHC. This indicated that the NIs had only short-term effects under all tested moisture levels. In contrast, Menéndez et al. (2012) and Torralbo et al. (2017) suggested that soil water content had minor effects on the persistence of DMPP in soil incubated with temperatures up to 20 °C. These contrasting results might be attributed to

Fig. 4 Relative abundance of selected nitrifying bacterial genera in an untreated (No-NI) and MP + TZ or DMPP treated silt loam soil fertilized with ammonium sulfate and subjected to different moisture conditions
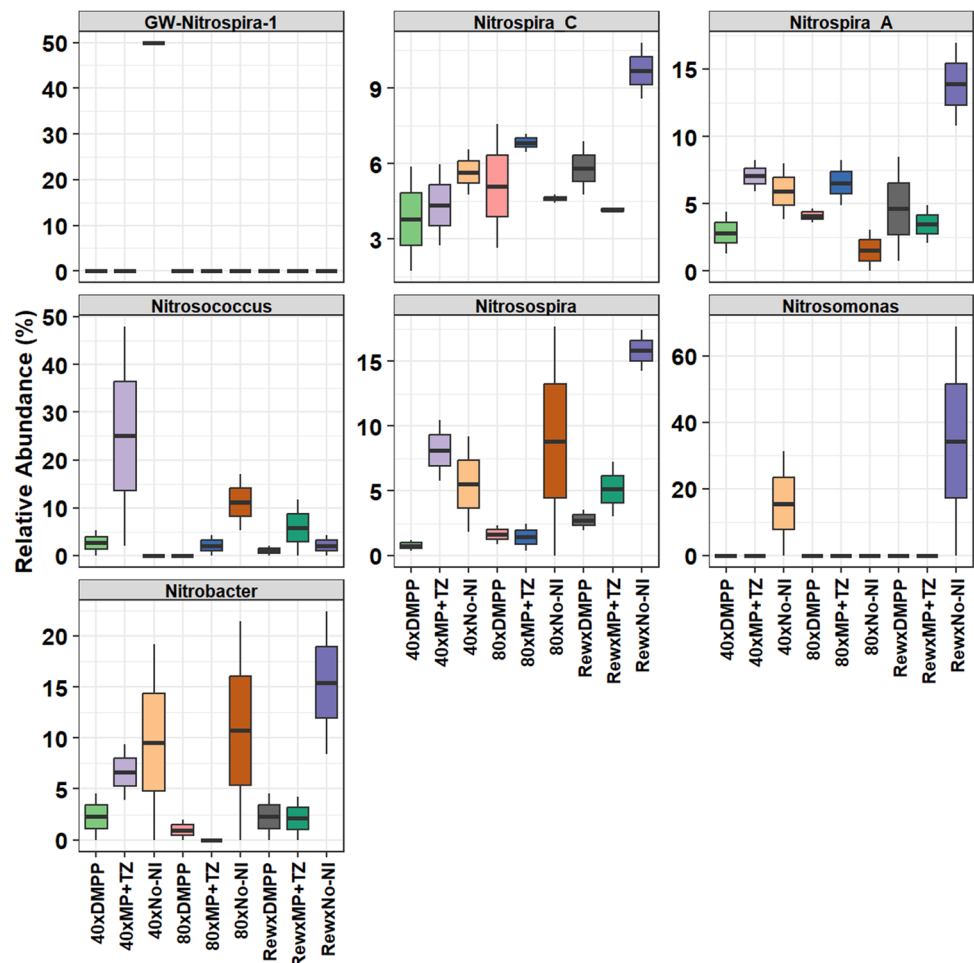
the higher temperature used in our study (25 °C) or distinct soil characteristics such as pH and texture.

The accumulation of NO_3^--N after 30 days in the silt loam soil under 80% WHC increased N_2O-N emissions by up to fivefold in DMPP and MP + TZ, respectively, relative to the No-NI treatment (Table 2). Consequently, this increased N_2O-N emissions in the NI-treated soils during Phase 2 partially offset the reduced emissions during Phase 1 (Fig. 2). In contrast, DMPP and MT + TZ did not increase N_2O-N release during Phase 2 in the sandy soil. Fine-textured soils commonly present higher microbial abundance and activity than coarse-textured ones (Barth et al. 2019; Guo et al. 2021), possibly decreasing NIs persistence in the silt loam soil.

It is widely known that NIs have a short-term effect as they are subjected to biodegradation. This effect may vary from 4 to 10 weeks depending on conditions such as soil temperature, texture, and pH (Zerulla et al. 2001). For instance, Menéndez et al. (2012) reported that DMPP caused a 45% and 23% reduction in N_2O-N emission in soil incubated under 10 °C and 20 °C, respectively, which suggested that higher temperature reduced the overall effectiveness

of DMPP. However, it has not been demonstrated yet that high soil water content could partially offset the short-term effect of NIs on reducing N_2O-N release (Fig. 2). Extensive research has investigated the interaction between NIs and different moisture levels (Menéndez et al. 2012; Guo et al. 2022; Zhang et al. 2023). Torralbo et al. (2017) and Zhang et al. (2023) reported that DMPP was effective under high soil water contents. However, its efficiency was more sensitive to increased temperature under such conditions. Guo et al. (2022) reported that MP + TZ efficiently mitigated nitrification and N_2O-N release under 80% WHC. In our study, the warm temperature in the incubation experiments (25 °C) probably stimulated NI biodegradation, and the hypoxic conditions under 80% WHC led to increased N_2O-N release due to late NO_3^--N accumulation.

Soil microbial activity and decomposition are known to have a parabolic relationship with moisture. Thus, it is expected that the biodegradation of SOC is minimal in extremely dry or wet conditions. At the same time, the highest biodegradation occurs at a soil moisture level where the water and O_2 availability are optimal (Dan et al. 2016). In our study, O_2 availability at 80% WHC was sufficient to

promote rapid nitrification in the No-NI treatment (Fig. 3). Similarly, the greater water availability at 80% WHC might have enhanced the biodegradation of NIs relative to the 40% WHC and drying-rewetting treatments. Even if this concept is well-known and described in the literature, our study is the first to show that this process can trigger increased N_2O-N emissions from NI-treated soil after 30 days. In contrast to our study, previous research found that the soil moisture did not influence or had only minor effects on NI effectiveness (Menéndez et al. 2012; Guo et al. 2022; Zhang et al. 2023). Consequently, at least for fine-textured soils with high pH under warm temperatures, our results are important to highlight that soil moisture should not be overlooked in future research or for using NIs in agriculture.

Soil texture differently affected the effectiveness of DMPP and MP + TZ

The mean reduction in N_2O-N emissions caused by DMPP and MP + TZ was 43% and 20%, respectively. This decrease in N_2O-N emissions by NIs is ascribed to their direct effect on nitrification and an indirect impact on denitrification due to diminished soil NO_3^- availability (Wu et al. 2017).

DMPP-induced decline in mean N_2O-N emissions was higher for sandy soil (46%) than silt loam soil (35%). Similarly, Barth et al. (2019) suggested that DMPP is more effective for coarse than fine-textured soils, probably because of adsorption on silt and clay particles associated with the enhanced microbial activity of fine-textured soils. Conversely, the mean decline in N_2O-N emissions caused by MP + TZ was lower in sandy soil (12%) than in silt loam soil (36%). In addition, MP + TZ significantly reduced N_2O-N release in the sandy soil only under drying-rewetting during Phase 1. These results showed that MP + TZ had inferior performance than DMPP in the sandy soil, which might be explained by a greater MP + TZ soil mobility, which may cause leaching and spatial separation from NH_4^+-N in coarse-textured soils. In contrast, Guo et al. (2021) found that the inhibitory effect of MP + TZ on N_2O-N release was greater in sandy than in clayey texture; however, the authors utilized a fivefold higher MP + TZ application rate than that used in this study. In addition, DMPP and NH_4^+-N are positively charged and, consequently, expected to present similar spatial distribution in the soil surface layers (Zerulla et al. 2001), which may explain the higher effectiveness of DMPP, especially in coarse-textured soils.

It is noteworthy that our study might have underestimated MP + TZ efficiency for two reasons. Firstly, we utilized the commercial product where the NI active ingredients were dissolved in urea ammonium nitrate solution. Therefore, the soil treated with MP + TZ received a slightly higher N rate. However, this variation is lower than 1% because the application rate of the commercial product containing MP + TZ was only 1% of the applied N. Secondly, the MP + TZ rate recommended by the manufacturer is probably based on lower N rates than the one utilized in our study. Despite this, MP + TZ delayed nitrification and reduced N_2O-N emissions in the silt loam soil (Figs. 2 and 3). Finally, MP + TZ efficiency in reducing N_2O-N release has been demonstrated in several studies under different environmental conditions (Fan et al. 2022; Guo et al. 2021; Pietzner et al. 2017; Scheer et al. 2017; Wang et al. 2020; Wolf et al. 2014; Wu et al. 2017).

The increased N_2O-N release in NI-treated soils after 30 days of incubations was observed exclusively in the silt loam soil (Table 2, Fig. 2). This soil had 30% higher WHC than the sandy soil, which probably led to enhanced microbial activity and biodegradation of the NIs, explaining such results.

DMPP and MP + TZ temporarily reduced the relative abundance of nitrifying bacteria

The treatments presented a similar average number of reads classified as bacteria in amplicon libraries, between 15,000 and 20,000 reads, and did not impact the Shannon index (Figures S1 and S2). Similarly, Barrena et al. (2017) and Corrochano-Monsalve et al. (2021b) indicated that DMPP did not impact the total bacterial abundance and Shannon index compared to control treatments, respectively. Such results evidence that DMPP and MP + TZ specifically target the nitrifying bacteria rather than affecting the total bacterial soil community. In addition, the effect of MP + TZ on soil microbial abundance has not been reported previously.

In general, the NIs decreased the relative abundance of the nitrifying bacteria 15 days after incubation (Figs. 4 and 6). Corrochano-Monsalve et al. (2021b) found that DMPP had a specific inhibitory effect on *Nitrosomonas* and did not affect other nitrifying bacteria genera. Alternatively, our results indicated that the relative abundance of *Nitrospira* spp., *Nitrosospira*, *Nitrosomonas,* and *Nitrosococcus* decreased due to DMPP and MP + TZ application. Similarly, Lan et al. (2022) demonstrated that DMPP application reduced the relative abundance of *Nitrosospira* and *Nitrosomonas* from acidic and calcareous soils. Li et al. (2023) found that DMPP reduces the gene abundance of ammonia-oxidizing bacteria (AOB) from different soil types. It is noteworthy that the bacterial DNA utilized to estimate the relative abundance of nitrifying bacteria may contain DNA from dead, inactive, or low-active microbes; thus, it might provide biased evidence of the bacterial genera driving the nitrification process (Nannipieri et al. 2019). Consequently, the interpretation of the data presented in this study should take it into account.

The exact mode of action of DMPP and MP + TZ is unknown (Bozal-Leorri et al. 2022). Nevertheless, it is

known that they inactivate the ammonia monooxygenase enzyme and inhibit the first step of nitrification (Corrochano-Monsalve et al. 2021a). Thus, the AOB population is commonly diminished by applying NI-treated N compared to conventional N fertilizers (Hayden et al. 2021). Accordingly, our results corroborated this premise. In addition, the positive correlation between N_2O-N release and *Nitrosospira* and *Nitrobacter* suggested that nitrifying bacteria were directly or indirectly responsible for producing this GHG in our study (Fig. 5).

At day 45, the inhibitory effect of the studied NIs weakened under all investigated moisture treatments. For instance, the relative abundance of *Nitrosococcus* increased considerably at day 45 relative to day 15 in NI-treated soils under 40% WHC, mainly in the MP + TZ treatment (Fig. 6). These results suggest that irrespective of the soil moisture condition, the effect of DMPP and MP + TZ on mitigating nitrification was short-term. Therefore, considering the medium to long-term impacts of NI application, increased N_2O-N emissions might be expected if conditions favorable to denitrification (anoxic environment, availability of organic C, and NO_3^-) are present regardless of anterior soil water content. In line with these results, Scheer et al. (2017) indicated that NIs can increase post-harvest N_2O-N release

due to late NO_3^- accumulation in the soil profile, which completely offset the lower emissions observed during the cropping period. Similarly, Guardia et al. (2023) reported that DMPP caused only a transitory reduction in the gene abundance of AOB in drip-irrigated soils.

These results are crucial for managing the application of NIs in agricultural systems. For instance, the delay in nitrification caused by NIs may promote the misleading interpretation that other N management practices, such as split applications, are unnecessary. However, under high soil water content and warm temperatures, such practices should be encouraged to avoid unintended high N_2O-N emissions in the medium to long-term crops. Accordingly, the N rate might also be adjusted based on the possibly higher nitrogen use efficiency promoted by NIs to prevent N surplus in soils.

Conclusions

Our findings did not support the hypothesis that soil drying and rewetting reduce the efficiency of NIs. Conversely, DMPP and MP + TZ applications effectively mitigate N_2O emissions upon soil rewetting within a 30 days period. Therefore, the results evidenced that applying NIs can be

Fig. 5 Correlation among relative abundance of selected nitrifying bacteria genera, N_2O-N emissions, and soil NH_4^+-N and NO_3^--N concentrations in an untreated (No-NI) and MP + TZ or DMPP silt loam soil fertilized with ammonium sulfate and subjected to different moisture conditions
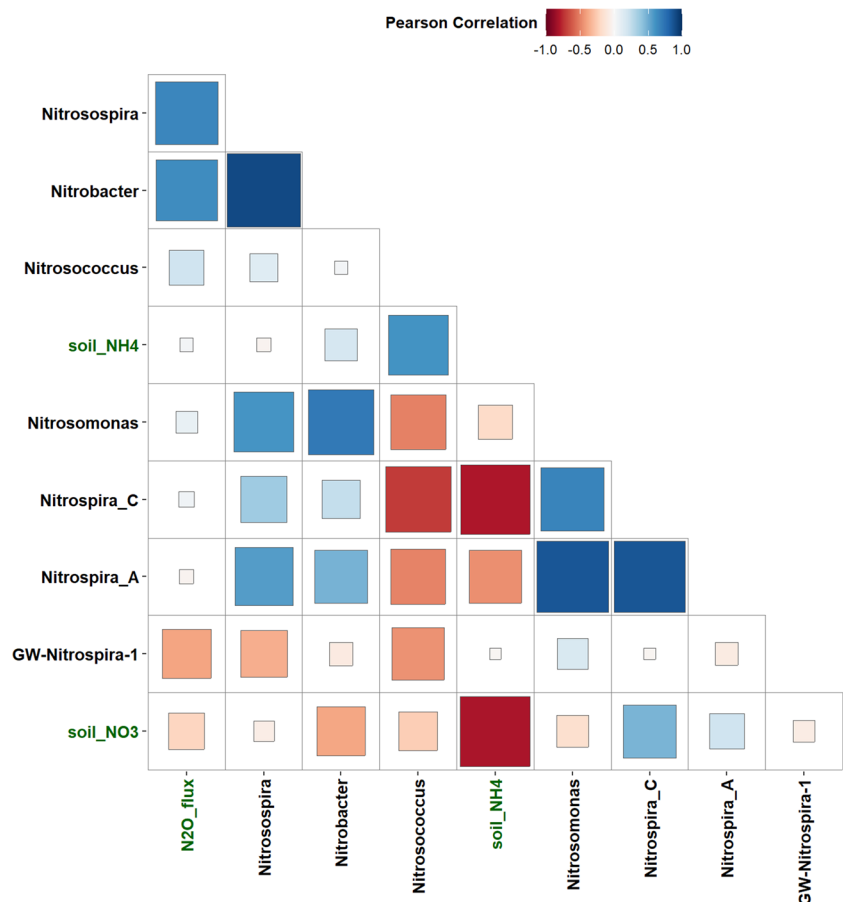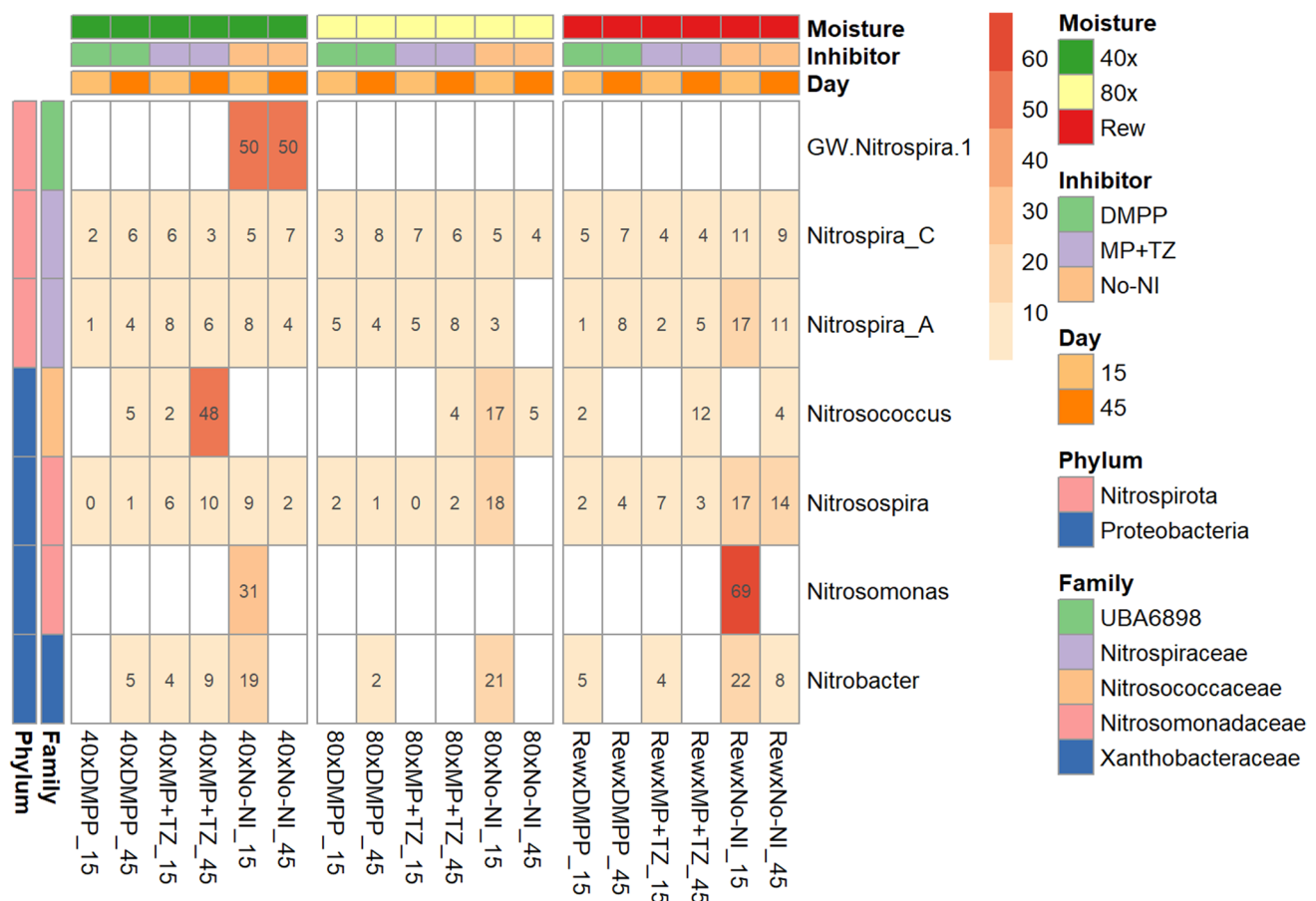
Fig. 6 Heatmap indicating the relation among the relative abundance of selected nitrifying bacterial genera and the tested treatments in an untreated (No-NI) and MP+TZ or DMPP treated silt loam soil ferti-lized with ammonium sulfate and subjected to different moisture conditions at 15 and 45 days after incubation

a proper mitigation measure for N₂O release in a scenario with more frequent soil drying and rewetting cycles due to climate change. Our second hypothesis that DMPP has a more stable effect than MP+TZ in delaying nitrification over different soil textures and moisture conditions was proved correct. MP+TZ presented a low capacity of inhibiting nitrification in the sandy soil and, therefore, it might not be recommended for coarse-textured soils, considering the conditions in which this study was carried out. In addition, this study indicated that, under favorable conditions to denitrification in the long-term, late high N₂O-N emissions from fine-textured soils treated with NIs may partially offset the short-term inhibition of nitrification. This result can promote more efficient planning of NI use in agriculture once the utilization of NIs may cause the misleading interpretation that other N management practices to avoid N surplus in soils are unnecessary. Finally, this is the first study to evaluate the effectiveness of NIs under soil drying and rewetting events, which are likely to occur more commonly in the future due to climate change. Therefore, further research is still necessary to investigate the effectiveness of NIs under more intense and/or repeated drying and rewetting cycles simulating extreme weather conditions for different agroecosystems.

Author contributions Pablo Lacerda Ribeiro and Karl Hermann Mühling contributed to the study conception and design. Material preparation, data collection and analysis were performed by Pablo Lacerda Ribeiro, Abhijeet Singh, Amit Sagervanshi and Asif Naeem. The first draft of the manuscript was written by Pablo Lacerda Ribeiro and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. Karl Hermann Mühling performed the project administration and funding acquisition.

Declarations

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