

The accumulation of mineral nitrogen in soil during drying events is affected by soil management

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ABSTRACT

Soil drying events play a critical role in shaping nitrogen (N) cycling in drylands by influencing N availability and the risk of N losses. This study examines how different soil management practices (no-tillage (NT) and traditional tillage (TT), with and without liming) affect N mineralization and the accumulation of inorganic N during drying periods in a hot-summer Mediterranean climate. Using soils from a long-term field experiment in southwestern Spain, we evaluated changes in inorganic N, enzymatic activities, and the abundance of genes related to N cycling during a summer fallow under field conditions, and in a complementary laboratory incubation under controlled temperature conditions. Field results showed a significant accumulation of inorganic N (from 6.47 to 11.43 mg N kg⁻¹) during drying, with a synergistic effect of NT and liming. Enzymatic activities (β -glucosaminidase, leucine aminopeptidase, proteases) and gene abundances (*chiA*, *pepA*, *apr*) declined with drying but remained higher under NT than TT. The laboratory study confirmed that management-induced differences in N cycling were mainly due to changes in soil biogeochemical properties (organic matter, pH), rather than changes in microclimatic conditions (soil temperature and moisture). While NT and liming enhanced N mineralization and microbial resilience, they also promoted inorganic N accumulation, increasing the potential for N losses (e.g., via N₂O emissions) upon rewetting. These results highlight the importance of integrating adaptive practices, such as summer cover cropping, into conservation agriculture to reduce N losses and improve nutrient use efficiency under increasingly frequent drought conditions.

1. Introduction

More than 40 % of Earth's terrestrial surface is covered by drylands or arid ecosystems, which together support more than a third of human population and play a key role in global biogeochemical cycling (Poulter et al., 2014; Osborne et al., 2022). Global climate change scenarios predict a modification of the rainfall pattern and a further warming of drylands, including more frequent and severe drought periods and heat waves followed by extreme precipitation events (Donat et al., 2016; Prävälíe, 2016). In consequence, it is expected that climate change will affect the biogeochemical processes relying on soil water availability as well as many other ecosystem services such as plant primary production, food production or greenhouse gases (GHGs) regulation (Austin et al., 2004; Delgado-Baquerizo et al., 2013; Prävälíe, 2016; Osborne et al.,

2022). In particular, soil drying strongly suppresses microbial activity and substrate diffusion, reducing the mineralization of organic matter and, consequently, nutrient availability (Borken and Matzner, 2009; Schimel, 2018).

The biogeochemical cycling of nitrogen (N) in drylands strongly depends on soil water availability which is affected by the alternation of dry-wet cycles caused by the seasonal nature of rainfall pulses (Austin et al., 2004; Delgado-Baquerizo et al., 2013; Zhang et al., 2023). One of the most sensitive processes to soil water availability is the mineralization of organic N in soils which can affect the N availability for plants (Schimel, 2018). It is estimated that more than 90 % of soil N is in organic form, predominantly (>70 %) occurring as proteinaceous compounds (Schimel and Bennett, 2004; Geisseler et al., 2010; Leinweber et al., 2013). Proteinaceous compounds including proteins,

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peptides, and amino acids are thus quantitatively important and biologically significant molecules associated with both C and N mineralization (Schimel and Bennett, 2004; Geisseler et al., 2010). The main bottleneck in N mineralization is depolymerization, which hydrolyzes large proteins and polypeptides into peptides and amino acids that can be directly taken up or further degraded to NH_4^+ (Jan et al., 2009; Geisseler et al., 2010). This step is primarily carried out by microbial-derived extracellular enzymes (Vranova et al., 2013) and based on the chemical composition of organic matter compounds hydrolyzed in soil. The most important depolymerases are proteases, chitinases and peptidoglycan hydrolases (Geisseler et al., 2010). In general, the activity of these N depolymerase enzymes is reduced during dry periods due to the lower substrate diffusion and enzyme degradation (Sardans and Peñuelas, 2010; Henry, 2012; Schimel, 2018). In addition, during dry periods, inorganic N (mainly NH_4^+ and NO_3^-) accumulates in the soil due to the low diffusion of ions and low N uptake by plant and microbial growth, however, the subsequent rewetting enhances the diffusion of the accumulated NH_4^+ and NO_3^- leading to accelerated nitrification and denitrification, reflected in N_2O emissions increase (L. Barton et al., 2013; Leitner et al., 2017; Vázquez et al., 2020a). These N_2O peaks after soil rewetting can be the main contributors to N_2O emissions in drylands (Mummey et al., 1994; Barton et al., 2011; Zhang et al., 2023). Therefore, understanding the processes involved in the production and accumulation of inorganic N during soil drying is crucial to avoid an intensification of N_2O emissions in drylands due to climate change.

While many studies have evaluated how the intensity and frequency of soil drying and rewetting affects the N mineralization and the accumulation of inorganic N in soils (Birch, 1958; Mummey et al., 1994; Austin et al., 2004; Borken and Matzner, 2009; Schaeffer et al., 2017; Homyak et al., 2018; Zhang et al., 2023), fewer studies have focused on how different soil management practices can affect the response of N cycling to soil drying. The popularity of agroecological practices and conservation agriculture is increasing in rainfed agriculture in drylands due to their capacity to increase soil organic matter and water availability in topsoil (Kassam et al., 2012; Serraj and Siddique, 2012; Aguilera et al., 2013). These changes in soil properties can lead to higher microbial activity and N mineralization in no-tillage (NT) systems as described by numerous authors (Oorts et al., 2006; Mahal et al., 2018; Vázquez et al., 2019a; Pecci Canisares et al., 2021), which in turn is often reflected in higher N uptake by plants and higher crop yields in conservation agriculture (Vázquez et al., 2020b; Pecci Canisares et al., 2021). However, during the dry periods, the higher soil moisture and organic matter in NT systems can increase N mineralization compared to tilled systems (Hueso et al., 2012; Vázquez et al., 2017). This higher N mineralization can trigger the inorganic N accumulation in soils during the dry periods when the plant and microbial N uptake is limited, and potentially, higher N losses as N_2O upon soil rewetting could be expected. This can be particularly critical in systems where NT management is combined with liming to raise the pH of acidic soils, as liming generally enhances N mineralization, which would otherwise be limited by low soil pH (Inagaki et al., 2016; Teutscherova et al., 2017; Vázquez et al., 2019b). While during the cropping season the combination of NT and liming increase the N available and uptake by crops (Caires et al., 2011; Vázquez et al., 2020b), during summer fallow periods it can lead to accumulation of inorganic N in soils. For example, in a study performed under rainfed conditions in Spain, Vázquez et al. (2019b) described a higher gross mineralization during the fallow period when NT was combined with liming because of the higher soil organic matter, soil pH and water availability compared to tilled soils. Therefore, the soil management practices adopted in drylands to enhance the soil water availability for crops and organic matter could, paradoxically, have the drawback of leading to a higher accumulation of inorganic N prone to losses during the dry fallow period unless appropriate agronomic measures are taken to prevent it.

Despite the potential implications of different management practices

on N cycling during soil drying, the availability of studies focused on this topic is scarce. One of the reasons is the difficulty for evaluating the N mineralization under dry conditions. The evaluation of the drying effects in soil enzymatic activity is problematic because classical potential enzymatic activity methods are evaluated under optimal experimental conditions hindering the effect of reduced substrate diffusion caused by low water availability under real conditions (Henry, 2012). In this sense, the estimation of selected genes encoding key enzymes responsible for the N mineralization process, such as *chiA* (chitinase), *pepA* (leucine aminopeptidase) or *apr* (protease) could help to further understand the effects of soil drying on the enzyme pool in soils (Nannipieri et al., 2012; Baraniya et al., 2016; Ouyang et al., 2018; Ouyang and Norton, 2020; Zhang et al., 2022).

Aiming to shed light on the impacts of soil management practices on the biogeochemistry of N cycling in drylands, we combined a field and a laboratory study to disentangle the role of soil management in N mineralization and inorganic N accumulation in soils during a drying event. The purpose of combining both approaches was to isolate, in the laboratory study, the microenvironmental conditions (soil moisture and temperature) resulting from different management practices from the soil's biogeochemical properties. First, we evaluated the N mineralization and the accumulation of inorganic N during summer fallow in a long-term field experiment comparing the effect of type of tillage (no-tillage (NT) vs traditional tillage (TT)) and liming. Second, we performed a soil drying experiment under controlled conditions at two temperature levels using soil from the same field experiment to distinguish the impacts of management practices on N mineralization and accumulation of inorganic N via changes in soil biogeochemical properties (soil organic matter, pH microbial populations, availability of organic and inorganic nutrients) and via soil microenvironmental conditions (soil water availability and temperature). In both studies, the accumulation of inorganic N as well as the activity of selected enzymes involved in N mineralization and the abundance of the encoding genes of these enzymes were examined. We hypothesized that:

- i) Soil drying leads to an accumulation of inorganic N (sum of $\text{NH}_4^+ - \text{N}$ and $\text{NO}_3^- - \text{N}$) in soils and limits the potential enzymatic activities related to N mineralization and the abundance of the genes encoding enzymes both under field and controlled conditions.
- ii) The different soil management practices affect the N mineralization and accumulation of inorganic N during soil drying events, particularly NT and liming can increase the N mineralization and accumulation of inorganic N during soil drying events
- iii) Higher effect of management practices on N mineralization and accumulation of inorganic N is expected during drying under field conditions than under controlled conditions because in field, these effects are caused not only by differences in soil biogeochemical properties but also by changes in soil microenvironmental conditions.

Understanding the impact of different management practices on N mineralization and inorganic N accumulation during dry periods is essential for developing management strategies that increase the N use efficiency and reduce the risk of N losses from agroecosystems.

2. Material and methods

2.1. Study site

A field experiment was established in 2005 on the detritic raña formation in Cañamero near Cáceres, SW Spain (39°19'N; 05°19'W; 588 m a.s.l.). The climate in the area is temperate with dry and hot summers (hot-summer Mediterranean climate, Csa) with a mean annual temperature of 16.3 °C, mean annual precipitation of 601.9 mm, and potential evapotranspiration (ETP) of 896.6 mm (Period 1981–2023, ERA5-

Land). The mean temperature for the summer period (June, July and August) is 26.1 °C, while accumulated rainfall and ETP for the summer period is 30.3 and 497 mm, respectively (Period 1981–2023, ERA5-Land). The soils are highly weathered, characterized by low pH and the exchange complex dominated by aluminum, and classified as Panthoskeletal Endoplastic Acrisols (IUSS Working Group WRB, 2015) or Typic Palexerult with a clayey-skeletal particle size class and kaolinitic mineralogy (Soil Survey Staff, 2022). Further details about the area, the dominant soils, and the vegetation can be found in (Vázquez et al., 2020c).

The experiment was designed as a split-plot design with four replicates and two studied factors: (i) tillage (NT vs. TT) and (ii) liming (amended vs. not-amended). Therefore, all the treatments included were traditional tillage without liming amendment application (TT), no-tillage without liming amendment application (NT), liming amended traditional tillage (TT-A) and liming amended no-tillage (NT-A). Each plot had a size of 64 m² (4 m x 16 m) and a total of 16 plots were established. Tilled treatments were ploughed with cultivator (20 cm depth) every autumn before sowing and the NT treatments have not been ploughed since 2005. As liming agent, we used a mixture of red gypsum and sugar foam (residual product of sugar industry), based on previous studies observing promising results of the used mixture for ameliorating soil acidity at both soil surface and subsurface (Gómez-Paccard et al., 2013). After the first application at the beginning of the experiment in 2005, refreshing doses of liming were applied in 2012 (2/7 of the initial application rate) and in October 2014 when 2.23 Mg ha⁻¹ of sugar foam and 4.28 Mg ha⁻¹ of red gypsum were applied to each plot (corresponding to 4/7 of the initial application rate, amount calculated for the period of four years) (Gómez-Paccard et al., 2015). The application of the refreshing dose of Ca-amendment was spread over the soil surface in both NT and TT plots and incorporated by tillage in case of TT treatment. Our study was conducted in this long-term experiment to better assess the cumulative effects of NT and liming on N dynamics over time, which may not be fully observable in short-term studies (Soane et al., 2012).

The samples for this study were collected in the agronomic season 2015/16. In November 2015 when the TT treatments were ploughed. A mixture of local varieties of *Avena sativa* L. (74 %) and *Vicia sativa* L. (26 %) was sown with a direct sowing machine after fertilization with 36 kg N ha⁻¹, 72 kg K ha⁻¹ and 72 kg P ha⁻¹. Crop biomass was harvested on the 31st of May 2016 and the field was left in summer fallow until the beginning of the next cropping season. Additionally, the soil temperature at a 5 cm depth was monitored using temperature loggers (DS1922L Thermochrom iButton, AlphaOmega Electronics; Madrid, Spain) by installing two loggers randomly in NT plots and other two in TT plots. More details on the applied management practices and experimental field design have been described in previous studies (Gómez-Paccard et al., 2013, 2015).

2.2. Field conditions experiment

Soil samples (0–10 cm depth) were collected on harvest day (May 31st, 2016) and in the middle of summer in the period of summer fallow (July 21st, 2016). During this summer fallow period, no crop or natural vegetation grows due to the water scarcity and the stubble from the previous cropping season is left on the soil surface. The daily air and soil temperatures and precipitation during this period is shown in Figure S1. During the study period (between the first sampling in May and the second sampling in July), the total rainfall of 30 mm was spread over seven rainfall events and the mean air temperature was 24.4 °C. The mean maximum air temperature was 33.4 °C during the two weeks before the second sampling point. The registered soil temperature at 5 cm depth during the whole study was 31.5 °C and the mean soil temperature during the last two weeks prior sampling were 21.7 °C and 36.6 °C in May and July, respectively.

In each sampling, a composite sample (ten sub-samples randomly

distributed across the plot area) was collected from each experimental plot. The soil samples were placed in plastic bags, transported immediately to the laboratory, and sieved to 2 mm on the same day. A sub-sample of each composite homogenized sample was stored at –20 °C for later DNA extractions and gene abundance quantification, one sub-sample was stored at 4 °C for potential enzymatic activity determination, and the rest of the soil was air-dried for chemical analysis. The basic soil chemical properties of the samples collected on both sampling dates are presented in Table S1. Further details about the sampling and the analytical methodology can be found in the companion paper where the same samples were used (Vázquez et al., 2019b).

2.3. Controlled conditions experiment

After the harvest (May 31st, 2016) additional composite soil samples were collected from the topsoil layer (0–5 cm) of each experimental plot. This depth was selected based on previous studies (Vázquez et al., 2019b) where most of the differences between TT and NT, as well as the effect of the surface-applied liming, were observed in the 0–5 cm soil layer. From each experimental plot (n = 16 as described in Section 2.1), 24 subsamples (2 mm-sieved, 10 g each) were lightly packed into incubation containers (250 ml) and soil moisture was adjusted to 60 % of the water holding capacity of each field experimental plot. Half of the 24 subsamples per plot were incubated at 25 °C (low temperature treatment) while the other half was incubated at (30 °C) high temperature treatment. Therefore, eight treatments combinations were studied: TT-low temperature, TT-high temperature, NT-low temperature, NT-high temperature, TT-A-low temperature, TT-A-high temperature, NT-A-low temperature and NT-A-high temperature with four replications per treatment combinations. The two temperatures were selected aiming to include two realistic levels of temperature slightly under and above the mean air temperature for the summer season in the region (26.1 °C).

After 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, 24, 26, and 29 days of incubation, one subsample was taken from each treatment combination to determine the concentration of soil exchangeable NH₄⁺ and NO₃⁻ making a total of 384 determinations. Additionally, a part of the samples collected on days 0, 17, and 24 was stored at –20°C for later DNA extractions and gene abundance quantification, and one subsample was stored at 4°C for potential enzymatic activity determinations. Soil moisture was monitored periodically during the incubation and samples were not rewetted allowing the soil drying. The 29-day incubation period and the sampling days for enzymatic analyses (0, 17, and 24) were selected based on the observed dynamics of inorganic N along the experiment, ensuring that key phases of N mineralization and accumulation were captured. This incubation experiment was finished after 29 days as the soil moisture was very low and no differences in soil moisture were observed upon day 24.

2.4. Laboratory analyses

The contents of NO₃-N and NH₄⁺-N in the soil samples from both the field conditions experiment and the controlled conditions experiment were determined by extracting mineral N from 5 g of fresh soil with 25 ml 1 M KCl, and quantified colorimetrically (UV-1203, Shimadzu, Kyoto, Japan) using methods proposed by (Miranda et al., 2001) and (Forster, 1995), respectively. The total inorganic N was calculated as the sum of NH₄⁺-N and NO₃-N concentrations.

In the field conditions experiment we determined the potential enzymatic activity of N-acetyl-β-D-glucosaminidase (β-glucosaminidase), leucine aminopeptidase, N-benzoyl-L-arginineamide protease (BAA-protease), casein-hydrolyzing enzymes (casein protease), L-asparaginase, L-glutaminase, urease and β-glucosidase. In the samples from day 0, 17 and 24 of the controlled condition experiment, we determined the potential enzymatic activity β-glucosaminidase, leucine aminopeptidase, BAA-protease and casein protease as these potential

enzymatic activities were the most affected by the sampling date in the field experiment, and thus, to soil drying (see Results section). All the determinations of potential enzymatic activities were performed using fresh soil following the protocols summarized in Table 1.

2.4.1. DNA extraction

Soil DNA was extracted from 0.25 g (total humid weight) of soil samples taken in May and July (field conditions experiment) and days 17 and 24 after incubation (controlled conditions experiment), using the PowerSoil DNA Isolation Kit (MoBIO Laboratories, Carlsbad, CA, USA), according to the manufacturer's instructions. DNA concentration and purity were determined by 260/280 nm and 260/230 nm measurements using a Nanodrop spectrophotometer (DeNovix, Wilmington, DE, USA).

2.4.2. Real-time PCR quantification

Quantitative PCR (qPCR) was performed to assess the abundance of the following genes: leucine aminopeptidase (pepA) and chitinase; GH 18 (ChiA) in both the samples from the field conditions experiments and controlled conditions experiment, while alkaline metalloproteinase (apr) was only determined in the field conditions experiment. The qPCR was performed in 20- μ L reaction mixtures containing the following components: 10 μ L of SYBR GreenER™ qPCR SuperMix (Invitrogen, NJ, USA), 0.4 μ M of each primer (resource Tables S2), and 4 μ L of diluted DNA extracts.

The optimal dilution of DNA extracts was tested to compensate for any reaction inhibition by humic acids co-extracted during DNA isolation (data not shown). All qPCR assays were run on an Applied Biosystems (Applied Biosystems, NJ, USA) ABI 7300 sequence detection system starting with an initial denaturation step at 95 °C for 10 min, followed by amplification cycles specific for each target gene (Table S2). A melting curve analysis was performed after each assay to ensure that only the products of the desired melting temperature were generated from the SYBR Green qPCR. The R2 values for the standard curves were 0.99 or higher in all runs. The standard curves for quantifying gene copy numbers were determined by cloning the PCR products. The population sizes of apr, pepA, and ChiA were estimated as the normalized copies per gram of dry soil.

The selection of chitinase (chiA), leucine aminopeptidase (pepA), and alkaline protease (apr) in this study was based on their key role in N mineralization and complements previous research studies on enzymatic activities performed in the same long-term experiment (Vázquez et al., 2019a, 2017). These enzymes are directly involved in the degradation of organic N compounds, facilitating their transformation into plant-available forms (Baraniya et al., 2016; Ouyang et al., 2018;

Table 1

Name of the enzyme, substrate used, product measured and the reference for each of the eight potential enzymatic activities determined in the soil samples.

Enzyme	Substrate used	Product measured	Ref.
β -glucosaminidase	p-nitrophenyl-N-acetyl- β -D-glucosaminide	p-nitrophenol	(Parham and Deng, 2000)
leucine aminopeptidase	L-leucine-4-nitroanilide	p-nitroanilide	(Daughtridge et al., 2021)
BAA protease	N-benzoyl L-arginine amide	NH_4^+ -N	(Ladd and Butler, 1972)
casein protease	sodium caseinate	Tyrosine	(Ladd and Butler, 1972)
L-asparaginase	L-asparagine	NH_4^+ -N	(Frankenberger and Tabatabai, 1991a)
L-glutaminase	L-glutamine	NH_4^+ -N	(Frankenberger and Tabatabai, 1991b)
urease	urea	NH_4^+ -N	(Kandeler and Gerber, 1988; Kandeler et al., 1999)
β -glucosidase	β -glucose-saligenin	Saligenin	(Strobl and Traunmueller, 1996)

Ouyang and Norton, 2020; Zhang et al., 2022). Chitinase catalyzes the breakdown of chitin, a significant N-containing polymer in soils, while leucine aminopeptidase and alkaline protease contribute to protein and peptide degradation, essential processes in nitrogen cycling.

2.5. Statistical analysis

The field experiment data were evaluated using a full factorial linear model considering the tillage type (TT vs NT), liming addition, sampling date (May or July) and their interactions as fixed factors, while the field block was considered a random factor. Because data were not normally distributed (Shapiro-Wilk-test, $p > 0.05$) all variables were log transformed prior to running the analysis.

For the incubation experiment, full factorial lineal models were used considering the tillage type, liming addition, sampling day, incubation temperature and their interactions as fixed factors. For the evaluation of soil inorganic concentrations and water content, 11 sampling days were included in the model (0, 1, 3, 5, 8, 10, 12, 15, 17, 24 and 29), while for the evaluation of the enzymatic activities and gene abundances only sampling days 17 and 24 were included in the model. Because data were not normally distributed (Shapiro-Wilk-test, $p > 0.05$) all variables were log transformed prior to run the analysis. Additionally, the correlation between the potential enzymatic activities and the abundance of encoding genes was performed using the Pearson's correlation test. The abundance of encoding genes was log-transformed before running the Pearson's test.

3. Results

3.1. Field conditions experiment

The concentration of the total inorganic N (the sum of NH_4^+ -N and NO_3^- -N concentrations) in soil increased significantly between May and July from 6.47 to 11.43 mg N kg^{-1} soil (Fig. 1; Table S3). No effect of tillage practice or liming was observed on the concentration of inorganic N, however we observed a significant interaction between tillage type and liming indicating a synergistic effect of increased inorganic N concentration in soil when lime is applied to no-tillage plots (Fig. 1; Table S3). No interaction between sampling date and tillage type or liming was observed.

The activity of most of the studied potential enzymatic activities (β -glucosaminidase L-asparaginase, L-glutaminase, urease and β -glucosidase) was higher in NT than in TT treatment (Fig. 2; Table S3). A similar

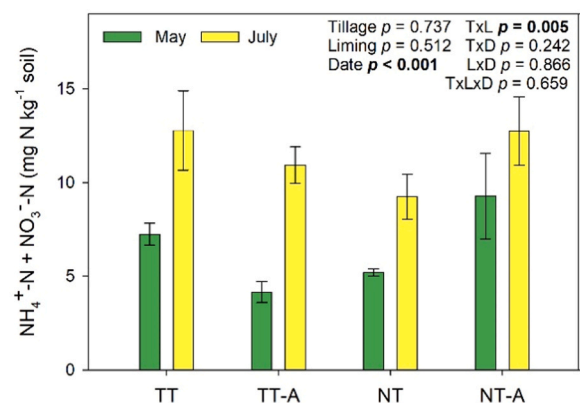


Fig. 1. Sum of NH_4^+ -N and NO_3^- -N concentrations at the samplings in May and July of soil samples (0–10 cm) from the field conditions experiment. Bars indicate means with standard error of the mean ($n = 4$). TT, Traditional tillage; TT-A Traditional tillage with liming amendment; NT, no-tillage; NT-A, no-tillage with liming amendment. In the upper right corner, the significance of the effects of tillage type (T) (no tillage or traditional tillage), liming addition (L), and sampling date (D) (May or July) and their interactions is shown.

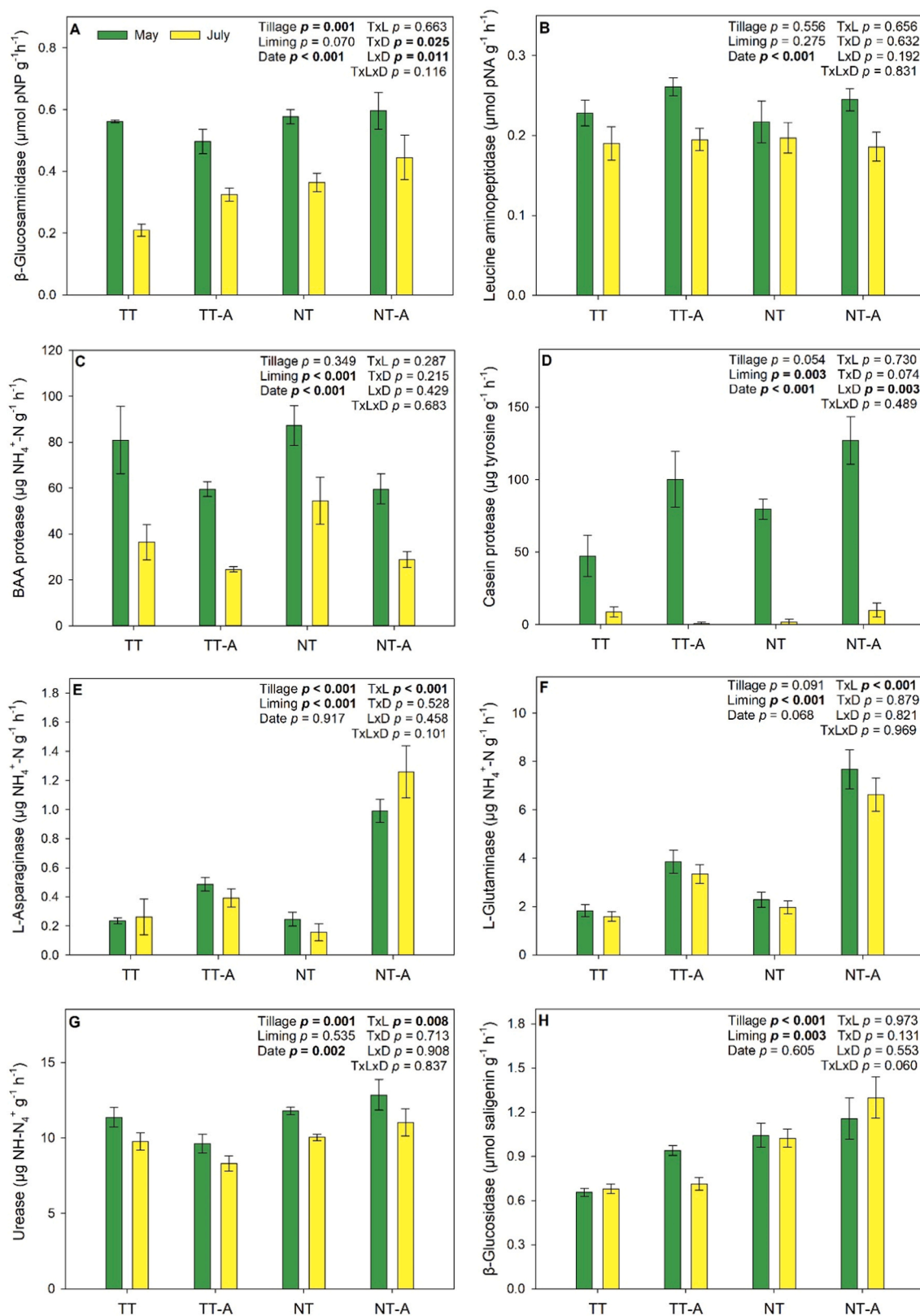


Fig. 2. Potential activity of β - glucosaminidase (A), leucine aminopeptidase (B), BAA protease (C), casein protease (D), L-asparaginase (E), L-glutaminase (F), urease (G) and β - glucosidase (H) at the samplings in field conditions experiment May and July of soil samples (0–10 cm). Bars indicate means with standard error of the mean ($n = 4$). TT, Traditional tillage; TT-A Traditional tillage with liming amendment; NT, no-tillage; NT-A, no-tillage with liming amendment. In the upper right corner, the significance of the effects of tillage type (T) (no tillage or traditional tillage), liming addition (L), and sampling date (D) (May or July) and their interactions is shown.

trend was observed in casein protease although the effect of tillage type was not significant ($p = 0.054$) (Fig. 2; Table S3). Only β -glucosaminidase showed a significant interaction between tillage type and sampling date indicating that the differences between NT and TT were higher in July than in May (Fig. 2; Table S3).

The effect of liming on the potential activity of the evaluated enzymes differed, showing a significant increase of the potential activity with liming addition compared to control for casein protease, L-asparaginase, L-glutaminase and β -glucosidase, and in contrast, significant negative effect of liming was observed in BAA protease (Fig. 2; Table S3). In addition, a significant interaction between tillage type and liming was observed for L-asparaginase, L-glutaminase and urease indicating that the combination of NT and liming synergistically increase the potential activity of these enzymes (Fig. 2; Table S3).

Most of the potential enzymatic activities determined (all except L-asparaginase, L-glutaminase and β -glucosidase) decreased significantly from the sampling in May to the sampling in July (Fig. 2; Table S3). Only one significant interaction between tillage type and sampling date was observed for β -glucosaminidase, indicating greater differences between tillage type in July than in May (Fig. 2; Table S3). A significant interaction was found between liming and sampling date for β -glucosaminidase and casein protease although the meaning of the interaction was the opposite in both enzymes (Fig. 2; Table S3).

No significant effect of tillage type or liming was observed in the three genes quantified in the soil samples from the field experiment sampling (*chiA*, *pepA* and *apr*) (Fig. 3; Table S4). However, the abundance of genes was strongly reduced between May and July samplings (Fig. 3; Table S4). The log-transformed abundance of *apr* was positively correlated with the potential activity of BAA and casein protease ($r = 0.443$, $p = 0.011$ and $r = 0.383$, $p = 0.030$, respectively, Table 2) and log-transformed *chiA* abundance was positively correlated with the potential activity of β -glucosaminidase ($r = 0.398$, $p = 0.024$, Table 2).

The soil water content in the samples collected for the field conditions experiment decreased from 10.8 % in May to 1.7 % sampling in July in average for the four treatments (Table S1). No significant differences between management practices on soil water content were observed (Table S1). The SOC concentration in NT was significantly higher than in TT and a similar trend was observed for the TN concentration (Table S1). Liming increased the soil pH from 4.98 in the control samples to 6.09 in the limed ones in average for both samplings and tillage practices (Table S1).

3.2. Controlled conditions experiment

The $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations followed a contrasting pattern during the incubation period, with the highest $\text{NH}_4^+\text{-N}$ concentration observed at the beginning of the incubation while the $\text{NO}_3^-\text{-N}$ concentration peaked on days 15 and 17 (Fig. 4; Table 3). The concentration of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and the total inorganic N was significantly higher in the NT than in the TT treatments (Fig. 4; Table 3). Similarly, the $\text{NO}_3^-\text{-N}$ and the total inorganic N concentrations were significantly increased by liming compared to the control (Fig. 4; Table 3). No significant interaction between tillage type and liming was observed (Fig. 4; Table 3). Only the $\text{NH}_4^+\text{-N}$ concentration was significantly affected by the incubation temperature showing higher concentrations at 30 than at 25 °C (Fig. 4; Table 3).

The four potential soil enzymatic activities determined in the controlled conditions experiment (β -glucosaminidase, leucine aminopeptidase, BAA protease and casein protease) were significantly higher in NT than in TT (Fig. 5; Table 4). Liming significantly increased β -glucosaminidase, leucine aminopeptidase and casein protease while reduced BAA protease compared to control (Fig. 5; Table 4). Only casein protease was affected by the sampling date showing a higher activity in day 17 of incubation (Fig. 5; Table 4). The incubation temperature only significantly affected the activity of BAA protease which was higher at 25 than a 30 °C (Fig. 5; Table 4). However, the interaction between

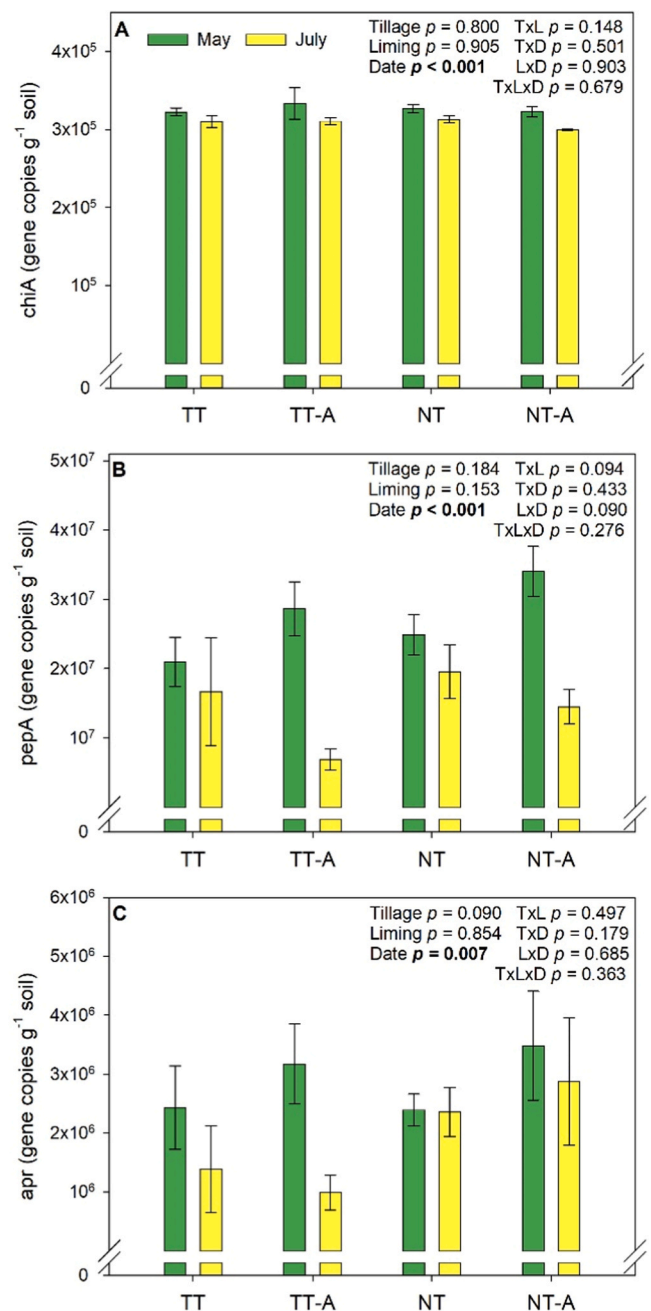


Fig. 3. Abundance of *chiA* (A), *pepA* (B) and *apr* (C) genes at the samplings in May and July of soil samples (0–10 cm) from the field conditions experiment. Bars indicate means with standard error of the mean ($n = 4$). TT, Traditional tillage; TT-A Traditional tillage with liming amendment; NT, no-tillage; NT-A, no-tillage with liming amendment. In the upper right corner, the significance of the effects of tillage type (T) (no tillage or traditional tillage), liming addition (L), and sampling date (D) (May or July) and their interactions is shown.

tillage type and incubation temperature indicated that the incubation at 30 °C increased the activity of β -glucosaminidase and leucine aminopeptidase in NT compared with the incubation at 25 °C while the higher temperature reduced the activity in TT compared to the lower temperature (Fig. 5; Table 4).

The abundance of *chiA* and *pepA* genes in soil samples was significantly higher in NT than in TT and on day 17 of incubation than in the day 24 (Fig. 6; Table 4). Liming increased the abundance of *chiA* compared to the control, while liming increased the abundance of *pepA* in NT but decreased it in TT (Fig. 6; Table 4). The interaction between

Table 2

Pearson correlation coefficients between potential enzymatic activities and corresponding gene abundance for the field and controlled conditions experiment.

Genes	Enzyme	r	p value	Experiment
chiA	β -glucosaminidase	0.398	0.024	Field
pepA	leucine aminopeptidase	0.332	0.064	Field
apr	BAA protease	0.443	0.011	Field
apr	casein protease	0.383	0.030	Field
chiA	β -glucosaminidase	0.422	0.001	Controlled
pepA	leucine aminopeptidase	-0.044	0.698	Controlled

The corresponding gene abundances were log-transformed before performing the Person's test.

tillage and temperature in the abundance of *chiA* indicated that the incubation at 30 °C increased the abundance of *chiA* in NT compared with the incubation at 25 °C, while the higher temperature reduced the abundance in TT compared to the lower temperature (Fig. 6; Table 4). The log-transformed abundance of *chiA* was positively correlated with the potential activity of β -glucosaminidase ($r = 0.422$, $p < 0.001$, Table 2).

The water content decreased significantly every day (except between days 24 and 29) during the controlled conditions experiment, and the decrease was stronger in the high incubation temperature (30 °C) compared to the lower incubation temperature (25 °C) (Fig. 4; Table 3). In addition, the interaction between tillage type and incubation temperature indicated that the water content was higher in NT than in TT at 25 °C over the whole experiment, while no differences were observed at 30 °C (Fig. 4; Table 3).

4. Discussion

Our study showed that different soil management practices can affect N mineralization and accumulation of inorganic N during the soil drying events. As more frequent and longer drought periods are expected to occur in the future, the understanding of the triggers leading to enhanced N accumulation is of a crucial importance to avoid or reduce the N₂O emissions peaks after soil rewetting (Mummey et al., 1994; Barton et al., 2011; Leitner et al., 2017; Zhang et al., 2023). Thus, the impact of soil management practices on inorganic N accumulation during dry periods must be also considered when selecting the most appropriate agronomic management practices for each region.

4.1. Effect of soil drying and management practices on inorganic nitrogen accumulation

Inorganic N accumulated in soils during soil drying both in the field and under controlled conditions experiments (Fig. 1, Fig. 3). Two main mechanisms can explain the inorganic N accumulation in soil during the field conditions experiment; 1) the absence of plant growth and therefore plant inorganic N uptake during the summer fallow, and 2) the low microbial N uptake (N immobilization) because the low soil water content limits microbial growth and causes microbial dormancy or death (Parker and Schimel, 2011; Schaeffer et al., 2017; Schimel, 2018). Similar mechanisms also likely play role under controlled conditions where plants were absent, and the soil water content strongly decreased during the incubation period (Fig. 4). Further, we discuss the differences between both experimental approaches in detail.

A synergistic effect of combining NT and liming on the accumulation of inorganic N was observed in the field conditions experiment (Fig. 1). The same synergistic effect of both practices was also observed in case of potential activity of L-asparaginase, L-glutaminase and Urease in this study (Fig. 2) and in case of gross N mineralization rates determined in this experiment during the same period in the parallel paper (Vázquez et al., 2019b). This suggests that although SOC and total N accumulated equally in all the NT treatments (regardless lime application) (Table S1),

the low pH of the non-limed treatments hampered the N mineralization as described in another study using the same soil (Teutscherova et al., 2017). Therefore, the combination of NT and liming can stimulate the N mineralization synergistically due to the higher soil pH and total soil N, and thus increase the N accumulation during the summer fallow. This could be considered as a drawback of the NT and liming combination since the accumulated inorganic N is prone to N losses upon rewetting (Barton et al., 2011; Leitner et al., 2017; Vázquez et al., 2020a). In contrast, during the cropping season the higher inorganic N production can support a higher plant growth and N uptake compared to the other management (Vázquez et al., 2020b). Therefore, land managers and farmers should consider additional managements practices aiming to reduce this accumulation of inorganic N during summer fallow when NT and liming are combined. For example, the establishment of summer cover crops instead of fallow periods could catch the inorganic N that accumulates during soil drying.

Under controlled conditions where the differences between treatments in soil microenvironmental conditions were removed (soil water content was adjusted for all the samples and all the treatments had the same temperature), NT and liming increased the accumulation of inorganic N independently but not synergistically (Fig. 3). These similar results in both experiments do not confirm our third hypothesis of expecting higher differences between management practices in the experiment under field conditions than under controlled conditions due to the differences caused by microenvironmental conditions (higher soil water content and lower soil temperature in NT). Additionally, the lack of effect of incubation temperature (and the consequent differences in water content) on inorganic N accumulation during the controlled conditions confirms that small differences in temperature (+5 °C) and soil water content do not change significantly the inorganic N accumulation during drying. In conclusion, our results showed that the differences caused by NT and liming on inorganic N accumulation during drying are caused by their changes in soil biogeochemical properties rather than by their changes in soil microenvironmental conditions.

During the controlled conditions experiment, the NH₄⁺-N and NO₃-N concentrations followed a contrasting pattern along the incubation period: the highest NH₄⁺-N concentration was observed at the beginning of the incubation while the NO₃-N concentration peaked at day 15 and 17 (Fig. 4). This indicates that despite the decreasing soil water content during the incubation, the nitrification process was active enough to transform most of the NH₄⁺ to NO₃. This partially contradicts previous studies showing a limited nitrification during the dry periods and an accumulation of NH₄⁺ (Parker and Schimel, 2011; Schaeffer et al., 2017), and the results obtained in the field conditions experiment of this study where N accumulates mostly as NH₄⁺-N during the summer fallow (Table S1 and Vázquez et al. 2019b). We hypothesize that despite the rapid reduction of soil water content during the incubation experiment, the water content was still high enough to allow the nitrification of the produced NH₄⁺ until approximately the 15th day of incubation when the soil water content decreased below 5 %. Upon the 15th day of incubation, the NO₃-N concentrations decreased indicating that the production of NO₃ was lower than the consumption (i.e., no net nitrification occurred). This water content of 5 % observed in our study as the limit for active nitrification in soil is similar to the "drought threshold" of 8 % of water content set as the minimum to activate the nitrification in Californian grasslands (Schaeffer et al., 2017). In addition, we observed that the concentration of NH₄⁺-N was higher at 30 than a 25 °C while the NO₃-N or total inorganic N were unaffected. This suggests that the N mineralization was not affected significantly by the temperature (or faster drying), but instead, the nitrification was limited by higher temperature (or faster drying) allowing a higher accumulation of NH₄⁺-N. Altogether, our results suggest that nitrification process is more sensitive than N mineralization to soil drying, and that depending on the speed of drying, the inorganic N accumulates as NH₄⁺-N (quicker drying) or as NO₃-N (slower drying). This assumption helps to reconcile the contrasting results found in literature where N accumulates as NH₄⁺-N

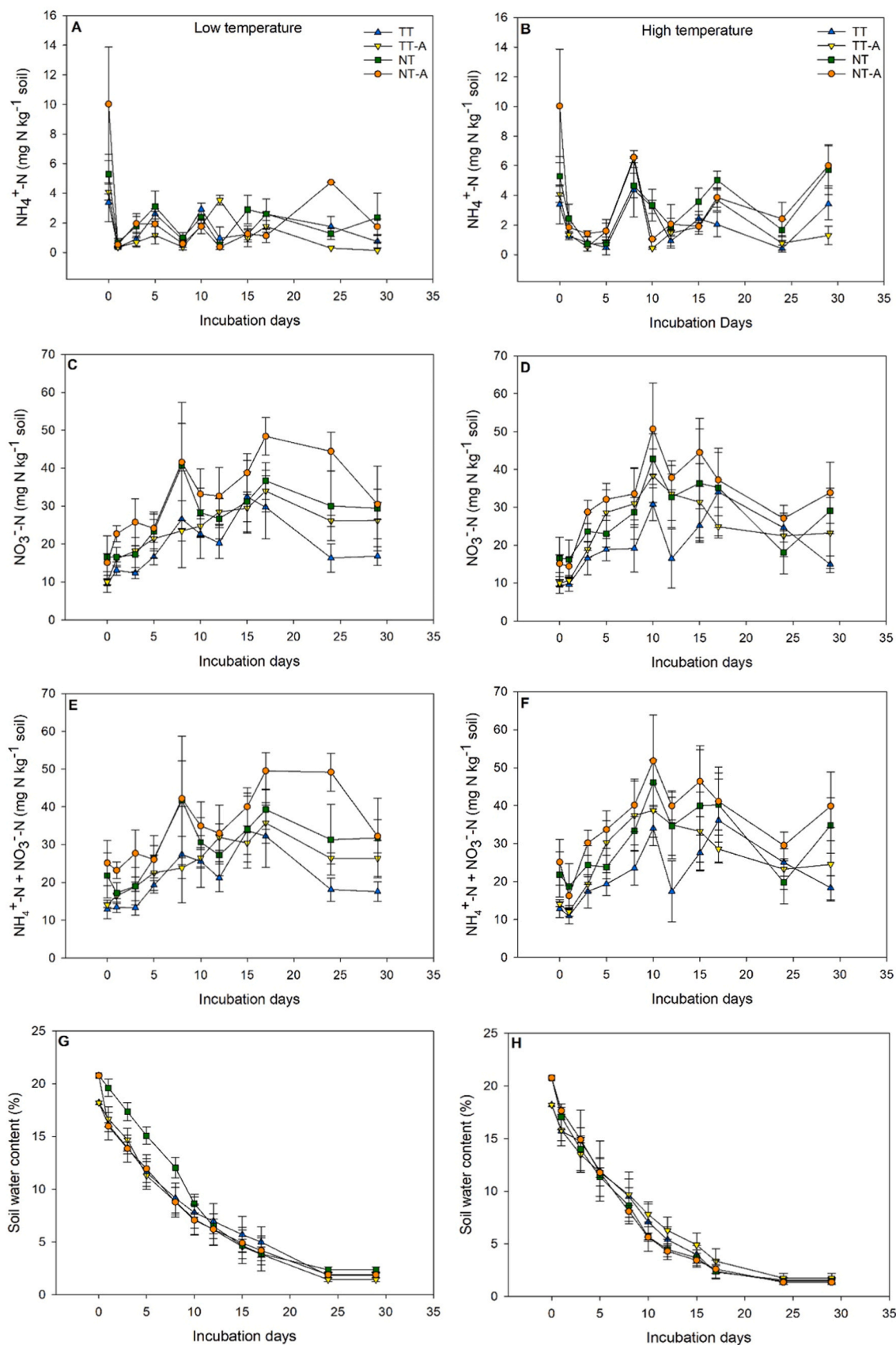


Fig. 4. Evolution of NH₄⁺-N (A, B), NO₃⁻-N (C, D) and the sum of NH₄⁺-N and NO₃⁻-N (E, F) concentration and soil water content (G, H) during the controlled conditions experiment at low and high temperature (25 and 30 °C, respectively) of soil samples (0–5 cm). Bars indicate standard error of the mean (n = 4). TT, Traditional tillage; TT-A Traditional tillage with liming amendment; NT, no-tillage; NT-A, no-tillage with liming amendment. The effect of tillage type (no tillage or traditional tillage), liming addition, sampling day (11 sampling points), incubation temperature (25 or 30 °C) and their interactions is shown in Table 3.

Table 3

The effect of tillage type (no tillage or traditional tillage), liming addition, sampling day (11 sampling points), incubation temperature (25 or 30 °C) and their interactions on the concentration of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and $\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$, and soil water content in samples from the controlled conditions experiment.

	$\text{NH}_4^+\text{-N}$		$\text{NO}_3^-\text{-N}$		$\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$		Water content	
	F	p	F	p	F	p	F	p
Tillage	30.07	< 0.001	42.06	< 0.001	51.03	< 0.001	0.23	0.630
Liming	0.29	0.590	20.49	< 0.001	20.08	< 0.001	1.34	0.248
Day	18.09	< 0.001	15.62	< 0.001	13.51	< 0.001	214.9	< 0.001
Temperature	28.33	< 0.001	0.09	0.767	0.13	0.714	16.40	< 0.001
Tillage x Liming	0.50	0.481	0.01	0.981	0.03	0.870	1.00	0.318
Tillage x Day	3.42	< 0.001	0.34	0.969	0.49	0.895	0.58	0.826
Tillage x Temperature	2.24	0.135	0.05	0.831	0.09	0.771	5.98	0.015
Liming x Day	3.68	< 0.001	0.40	0.946	0.49	0.897	0.07	1.000
Liming x Temperature	2.76	0.098	0.24	0.627	0.21	0.646	6.69	0.010
Day x Temperature	12.33	< 0.001	1.64	0.097	1.28	0.243	0.88	0.555
Tillage x Liming x Day	2.14	0.022	0.45	0.918	0.50	0.887	0.10	1.000
Tillage x Liming x Temperature	0.11	0.737	0.01	0.951	0.01	0.975	0.01	0.985
Tillage x Day x Temperature	1.28	0.244	0.80	0.625	0.86	0.572	0.33	0.973
Liming x Day x Temperature	2.09	0.026	0.41	0.941	0.48	0.903	0.12	1.000
Tillage x Liming x Day x Temperature	1.421	0.171	0.30	0.981	0.24	0.993	0.42	0.934

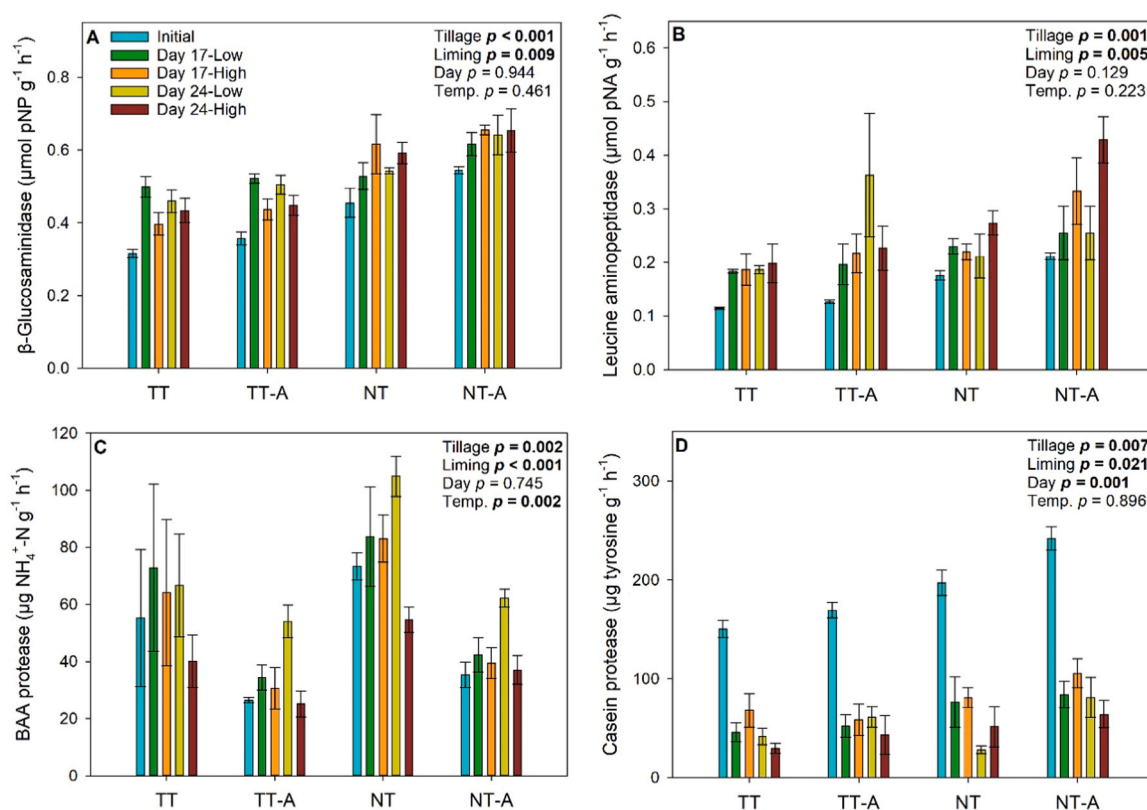


Fig. 5. Potential activity of β -glucosaminidase (A), leucine aminopeptidase (B), BAA protease (C) and casein protease (D) at day 0 (initial), 17 and 24 of the controlled conditions experiment at low and high temperature (25 and 30 °C, respectively) of soil samples (0–5 cm). Bars indicate means with standard error of the mean ($n = 4$). TT, Traditional tillage; TT-A Traditional tillage with liming amendment; NT, no-tillage; NT-A, no-tillage with liming amendment. In the upper right corner, the significance of the effects of tillage type (no tillage or traditional tillage), liming addition, sampling day (17 or 24 days of incubation) and incubation temperature (25 or 30 °C) is shown. The effects of treatment interactions are presented in Table S5.

(Parker and Schimel, 2011; Schaeffer et al., 2017) or as $\text{NO}_3^-\text{-N}$ (Barton et al., 2011, 2013; Vázquez et al., 2019c). In conclusion, our results suggest that in a scenario of increasing temperatures, soil drying could happen faster during the dry periods and thus, the inorganic N would accumulate in soil as $\text{NH}_4^+\text{-N}$ rather than as $\text{NO}_3^-\text{-N}$ which might reduce the risk of N losses upon rewetting.

4.2. Soil drying and management practices affected the potential enzymatic activities

Most of the measured potential enzymatic activities (all except L-asparaginase, L-glutaminase and β -glucosidase) decreased significantly between May and July indicating a negative effect of the summer fallow in the field conditions experiment (Fig. 2). In addition, casein protease was affected by soil drying in the controlled conditions experiment showing lower values at the end of the incubation period (Fig. 5). These results confirm our first hypothesis of soil drying limiting the potential

Table 4

The effect of tillage type (no tillage or traditional tillage), liming addition, sampling day (17 or 24 days of incubation) and incubation temperature (25 or 30 °C) and their interactions on the potential activity of β -glucosaminidase, leucine aminopeptidase, BAA protease and casein protease, and the abundance of *chiA* and *pepA* genes in soil samples from the controlled conditions experiment.

	glucosaminidase		leucine		BAA protease		casein protease		chiA		pepA	
	F	p	F	p	F	p	F	p	F	p	F	p
Tillage	58.86	< 0.001	7.60	0.008	11.26	0.002	7.85	0.007	19.30	< 0.001	7.55	0.008
Liming	7.48	0.009	8.51	0.005	23.72	< 0.001	5.74	0.021	7.87	0.007	0.38	0.542
Day	0.05	0.944	2.39	0.129	0.11	0.745	12.05	0.001	12.97	< 0.001	22.70	< 0.001
Temperature	0.55	0.461	1.52	0.223	10.86	0.002	0.02	0.896	1.04	0.313	0.67	0.418
Tillage x Liming	0.99	0.326	0.24	0.627	0.63	0.431	1.73	0.194	1.21	0.277	4.50	0.039
Tillage x Day	0.02	0.879	0.12	0.734	0.05	0.831	1.10	0.300	4.15	0.047	0.56	0.459
Tillage x Temperature	9.59	0.003	4.20	0.046	0.11	0.746	1.18	0.283	4.49	0.039	3.00	0.090
Liming x Day	0.01	0.908	1.19	0.280	1.62	0.210	2.46	0.123	1.46	0.233	0.03	0.861
Liming x Temperature	0.40	0.532	0.20	0.657	0.28	0.599	1.30	0.260	3.35	0.073	0.05	0.830
Day x Temperature	0.11	0.738	0.05	0.831	7.36	0.009	2.56	0.116	9.70	0.003	36.49	< 0.001
Tillage x Liming x Day	0.04	0.852	0.18	0.676	0.01	0.918	0.10	0.754	0.37	0.547	2.01	0.163
Tillage x Liming x Temperature	0.22	0.641	2.18	0.147	0.32	0.576	0.01	0.938	0.26	0.610	0.58	0.450
Tillage x Day x Temperature	1.29	0.262	2.52	0.119	0.02	0.885	1.35	0.252	2.44	0.125	0.56	0.460
Liming x Day x Temperature	0.09	0.763	0.26	0.459	0.01	0.938	0.56	0.457	3.03	0.088	0.15	0.701
Tillage x Liming x Day x Temperature	0.16	0.695	0.67	0.416	0.21	0.647	0.55	0.461	0.95	0.335	1.94	0.171

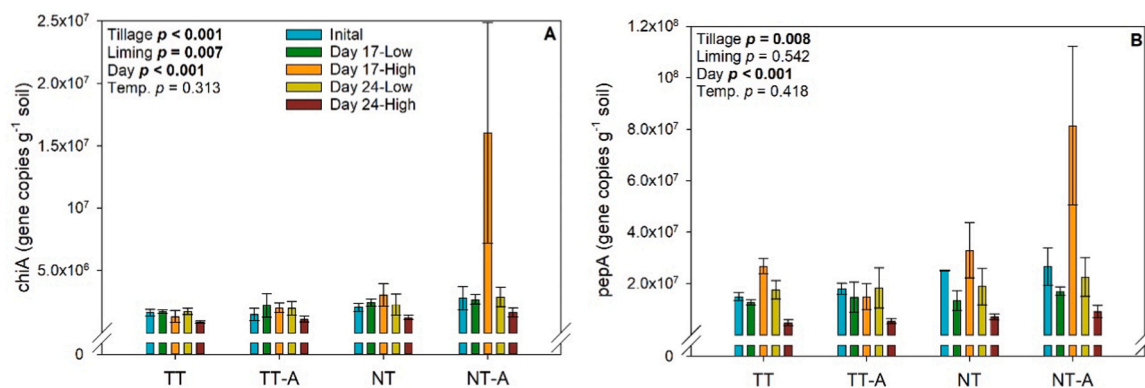


Fig. 6. Abundance of *chiA* (A) and *pepA* genes at day 0 (initial), 17 and 24 of the controlled conditions experiment at low and high temperature (25 and 30 °C, respectively) of soil samples (0–5 cm). Bars indicate means with standard error of the mean (n = 4). TT, Traditional tillage; TT-A Traditional tillage with liming amendment; NT, no-tillage; NT-A, no-tillage with liming amendment. In the upper left corner, the significance of the effects of tillage type (no tillage or traditional tillage), liming addition, sampling day (17 or 24 days of incubation) and incubation temperature (25 or 30 °C) is shown. The effects of treatment interactions are presented in Table S5.

enzymatic activities related to N mineralization. However, in this study we were particularly interested in the potential interaction between tillage type and sampling day or incubation temperature as it might suggest a different response to soil drying of the different evaluated management practices. In the field conditions experiment, a significant interaction between tillage type and sampling day was observed in case of β -glucosaminidase, indicating higher differences between TT and NT in July than in May (Fig. 2). In the controlled conditions experiment, the interaction between tillage type and incubation temperature indicated that the incubation at 30 °C increased the activity of β -glucosaminidase and leucine aminopeptidase in NT compared with the incubation at 25 °C while the higher temperature reduced the activity in TT compared to the lower temperature (Fig. 4). Both results in the field and controlled conditions experiment pointed towards a higher resistance of potential enzymatic activities to dryness and/or increasing temperatures in NT than in TT (Hueso et al., 2012; Vázquez et al., 2017). This can be caused by the higher soil organic matter in NT as well as the presence of stubble from the previous cropping over the soil surface, that can increase the soil water content and reduce the surface soil temperature compared to TT as previously described in Vázquez et al. (2017). In contrast to tillage type, no clear interactions between liming and the sampling day or incubation temperature were observed, indicating that liming does not affect N cycling during soil drying. In summary, our third hypothesis of management practices affecting the N mineralization

during soil drying was confirmed and particularly, NT seemed to ameliorate the effects of soil drying on some potential enzymatic activities related to N mineralization.

As previously mentioned, most of the measured potential enzymatic activities decreased with the advancing soil drying in both experiments (Fig. 2), however several considerations must be made. Firstly, the response to drying of the evaluated potential enzymatic activities differed depending on the specific enzymes: for example, while casein protease decreased 16 times and BAA protease 2 times between May and July in the field experiment, L-asparaginase and L-glutaminase were not affected by the sampling day. This contrasting impact of soil drying on different enzymatic activities could decouple the N mineralization as they represent different steps of N mineralization process: while proteases catalyze the key step of the decomposition of proteins in peptides and amino acids, L-Asparaginase and L-glutaminase catalyze the hydrolysis of amino acids into NH_4^+ . As the breakdown of proteins by protease is the main bottleneck in N cycle that limits further steps as ammonification and nitrification (Schimel and Bennett, 2004; Jan et al., 2009), our results suggest that the negative impact of soil drying on proteolytic activity may block the whole N mineralization and thus, hamper a further production and accumulation of inorganic N in drying soils. While this reduction of proteolytic activity by soil drying can be positive during fallow periods as it avoids the risk of N losses, it might decrease the N availability for crops in periods of water scarcity during

the cropping season. In their meta-analysis, Brzostek et al. (2012) found that the differences between precipitation and potential evapotranspiration during the growing season higher than 200 mm cause a decrease of proteolytic activity in experimental drought trials. However, more studies focusing on identifying the specific water content threshold that limits the proteolytic activity for each type of soil and ecosystem seems to be crucial to predict the impact of dry episodes on N availability for plants.

A second consideration is that soil drying affected more strongly the potential enzymatic activities in the field conditions experiment than in the controlled conditions experiment (β -glucosaminidase, leucine aminopeptidase, BAA-protease were affected by sampling day in the field experiment but not under controlled conditions) (Fig. 2, Fig. 5). This contrasting response of some of the potential enzymatic activity could be caused by the shorter extend/severity of soil drying under controlled conditions (24 days) compared to the field conditions experiment (51 days) or/and is a consequence of the limitations of the methodology used for determining the potential enzymatic activities. Regarding the severity of both drying experiment, in the study under controlled conditions, the temperature was set to 25 and 30 °C, while the soil temperature in the field experiment was on average above 35 °C during July reaching a peak of 44 °C on July 9th (Figure S1). These high soil temperatures registered during the field conditions experiment might have caused a higher enzyme denaturation compared to the controlled conditions experiment. Regarding the methodology used, the potential enzymatic activities are evaluated under optimal experimental conditions and represent the pool size of enzymes but does not account for limitations in the substrate diffusivity or availability (Henry, 2012; Nannipieri et al., 2018), thus, the impact of dry soils on *in situ* enzymatic activity due to low substrate diffusion is not accounted for. It is likely that the *in situ* enzymatic rates of these enzymes also decreased during the controlled conditions experiment due to the lower substrates mobility, however it was not detected by the selected methods for potential enzymatic activity that only accounted for the pool size of enzymes. In contrast, we can assume that the reduction of the potential enzymatic activity due the drying event in the field conditions experiment was due to a decrease of the pool size of β -glucosaminidase, leucine aminopeptidase and BAA-protease but not the drying event in the controlled conditions experiments.

4.3. Abundance of encoding genes was affected by soil drying but not by management practice

The abundance of the three encoding genes quantified in the field experiment (chiA, pepA and apr) was negatively affected by the sampling day indicating a negative effect of the summer fallow on their abundance (Fig. 3). Similarly, in the controlled conditions experiment the two quantified encoding genes (chiA, pepA) were significantly higher on day 17 than on day 24 (Fig. 6) confirming the field experiment observations. Furthermore, liming and NT increased the abundance of the two quantified encoding genes (chiA, pepA) in the controlled conditions experiment (Fig. 6), following the same pattern observed for the corresponding potential enzymatic activities. While the abundance of chiA gene was higher at 30 °C than at 25 °C in NT soil, the opposite was found in TT (gene abundance decreased with increasing temperature) (Fig. 6). This same pattern was observed in the potential activity of β -glucosaminidase (the enzymatic activity encoded by chiA). This suggests that NT increases the resilience to drying of both chiA and the potential activity of β -glucosaminidase (encoded by chiA gene) compared to TT, likely due to the higher soil organic matter accumulation in NT. This interaction confirms our second hypothesis of the different management practices affecting the N mineralization during soil drying as previously described also for the potential enzymatic activities. No effect of the management practices on the three encoding genes quantified in the field experiment (chiA, pepA and apr) was observed (Fig. 3) in contrast to the strong effect of tillage type and liming

observed in the controlled conditions experiment (Fig. 6). At the detection of a gene in the extracted soil DNA does not mean that the gene is being expressed (Nannipieri et al., 2018), in the field conditions experiment a part of the detected genes could be inactive due to the dry conditions. The determination of the gene transcription (synthesis mRNA) could help to assess the real enzymatic activity *in situ* (Nannipieri et al., 2018).

The decrease of the three encoding genes during the summer fallow in the field conditions study aligns with the results of the encoded enzymes that also decreased between May and July (Fig. 2, Table 2). The chiA gene encodes chitinase and has shown a good relationship with β -glucosaminidase enzyme (Ouyang et al., 2018), pepA encodes leucine aminopeptidase (Zhang et al., 2022), and apr encodes alkaline-metalloprotease and has shown good relationship with BAA-protease (Baraniya et al., 2016). In contrast, in the controlled conditions experiment, the abundance of the two genes (chiA and pepA) was affected by the sampling day but not the corresponding potential enzymatic activities (β -glucosaminidase and leucine aminopeptidase). This suggest that the quantification of the abundance of encoding genes was more accurate to evaluate the impact of soil drying on N mineralization dynamics than the determination of potential enzymatic activities, particularly in short-term studies like the controlled conditions experiment where the total pool of enzymes (including both the extracellular enzymes stabilized in the soil matrix and enzymes associated to microbes) was not affected by the severity of soil drying. These findings highlight the importance of integrating molecular and enzymatic approaches to better understand N mineralization under different management practices. Future research should explore the expression of these genes under fluctuating environmental conditions to further assess microbial resilience and adaptability in nitrogen-cycling pathways

4.4. Implications for soil management and nitrogen cycling

This study provides evidence that NT and liming significantly influence N cycling under soil drying conditions in Mediterranean agroecosystems. No-tillage enhances microbial resistance to drying by maintaining higher enzymatic activity than TT and sustaining N mineralization even under limited water availability. Meanwhile, liming increases N mineralization and inorganic N accumulation during dry periods by raising soil pH and alleviating soil acidity. In the presence of crops, the enhanced N mineralization resulting from NT and liming combination can improve plant N uptake and biomass production (Caires et al., 2011; Vázquez et al., 2020b). However, during fallow periods (common in Mediterranean regions due to low summer rainfall and high temperatures) this increased N mineralization promotes the accumulation of inorganic N, which may be lost through leaching or gaseous emissions upon rewetting. This can contribute to environmental pollution and thus should be avoided. Therefore, combining NT and liming with additional strategies is essential to prevent excessive N accumulation and losses. Potential solutions include using summer cover crops adapted to low water availability or incorporating perennial grain/forage crops that can survive the summer, replacing traditional annual crops. These alternative approaches could help maintain soil health and optimize N cycling under increasingly variable climate conditions and more frequent droughts.

5. Conclusions

This study assessed the impact of different soil management practices on N mineralization and inorganic N accumulation during soil drying, using a combination of experiments under field and controlled conditions. Soil drying promoted the accumulation of inorganic N and reduced the abundance of genes and enzymatic activities involved in N mineralization in both experiments. Gene abundance was more sensitive to the effects of drying than enzymatic activity, particularly under controlled conditions where drying was less intense.

The effects of soil management practices were consistent in both experiments and were mainly associated with changes in biogeochemical soil properties—such as organic matter and pH—rather than microenvironmental factors like temperature or water availability. Notably, NT increased microbial resistance to drying, as evidenced by higher enzymatic activity and gene abundance. When combined with liming, NT led to a synergistic accumulation of inorganic N under field conditions which suggest a risk of N losses upon rewetting.

These findings highlight the importance of considering soil management practices when evaluating N cycling under dry conditions, as excessive inorganic N accumulation increases the risk of losses upon rewetting. Furthermore, our results suggest that gene abundance may be a more sensitive indicator of drought effects than enzymatic activity. Complementary strategies such as summer cover cropping or perennial crops could help reduce N losses and improve N use efficiency in dryland systems.

CRedit authorship contribution statement

Teutscherová Nikola: Investigation. **Vázquez Eduardo:** Investigation. **Camara Joaquín:** Investigation. **Almorox Javier:** Investigation. **Kasschau Kristin:** Investigation. **Benito Marta:** Investigation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Marta Benito Capa reports a relationship with Universidad Politécnica de Madrid that includes: employment. If there are other authors, they 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. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.still.2025.106623](https://doi.org/10.1016/j.still.2025.106623).

Data availability

No data was used for the research described in the article.

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