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Factors influencing nitrogen derived from soil organic matter mineralisation: Results from a long-term experiment☆

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ABSTRACT

Mineralised nitrogen (N) from soil organic matter (SOM) is a crucial source of N for both natural ecosystems and agroecosystems. Therefore, accurate estimation of the amount of N available to crops from SOM mineralisation is necessary to correctly manage N addition. For application in an N budget, a field-scale assessment of the main factors affecting SOM mineralisation is required. The objective of this study was to quantify the influence of meteorological conditions and soil properties on N mineralised by SOM in an agroecosystem. The N mineralised from the SOM was calculated as the N uptake of the unfertilised plot minus the N derived from atmospheric deposition and irrigation. This study analysed 29 years of crop, agrometeorological, and soil data from three maize cropping systems (maize for grain, maize for silage, and maize-It. ryegrass double cropping) in a long-term experiment conducted in NW Italy. A Linear Mixed Model (LMM) was developed for the purpose of this study. The average of N derived from SOM mineralisation predicted by the model was 96 kg N ha^{-1} yr^{-1}, with a root mean square error of 22 kg N ha^{-1} yr^{-1}. The fixed factors of LMM, which are soil organic carbon (SOC), carbon-to-nitrogen ratio (C/N) and the sum of rainfall and irrigation (R.I.), were responsible for 19 % of the annual variations in mineralised N. SOC and R.I. had a positive effect and greater weight on the process, whereas C/N had a negative effect and lower weight. The explanatory power of the model increased to 52 % when cropping systems and interannual variability were included as random factors. This study highlights the importance of weather conditions and SOC content in determining the amount of N derived from soil mineralisation and can contribute to plant nutrition. In a future climate scenario characterised by increased aridity, N mineralisation could decrease, thus increasing the demand for fertilisers.

1. Introduction

Soil organic matter (SOM) is an important component of soil that consists of plant and animal residues, live and dead microbial biomass, by-products of microbial processes, and C coupled with mineral components as organo-mineral complexes (Lal, 2018). SOM mineralisation is a biological process by which microorganisms decompose SOM into its mineral components, or into smaller, simpler organic molecules when the mineralisation process is incomplete (Bridgham and Lamberti, 2009).

Nitrogen (N) released from SOM mineralisation is a crucial source of plant-available N and is critical for sustaining crop growth and productivity, not only in natural ecosystems (Cleveland et al., 2013), but also in agroecosystems (Johnston et al., 2009). Understanding this process is essential for optimising fertilisation management by synchronising N delivery with crop requirements (Fontaine et al., 2024). Knowing the amount of N that will be released from SOM mineralisation is of fundamental importance to calculate a reliable provisional nutrient management plan. This is a step towards decreasing fertiliser applications, as in the European Farm-to-Fork strategy (European Union, 2020).

The process of mineral N release from SOM decomposition is influenced by a multitude of variables, including SOM amount and type, soil properties, microbial activity, and meteorological conditions. The amount of SOM substrate influences the rate at which mineralisation occurs. In fact, SOM mineralisation is generally intended to be a first-order kinetic reaction, that is its rate is proportional to the reactant concentration (Manzoni and Porporato, 2009). To include the heterogeneity of SOM composition and following the classical, although not

☆ Tetto Frati dataset

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up-to-date vision (Lehmann and Kleber, 2015), SOM is generally described as composed of a set of pools with different degradability characteristics (Wander, 2004), such as labile, intermediate, and stable pools. Labile pools, with a half-life of several years, consist of easily degradable substrates such as microbial biomass components and litter residues. Intermediate pools, with a half-life ranging from a few years to decades, comprise partially-decomposed residues and some materials, such as mobile humic acids. Stable pools, with a half-life ranging from decades to centuries, consist of substrates that are difficult to degrade, such as aliphatic macromolecules, lignins, and humin (Wander, 2004). The total N content of the soil, which includes all organic fractions plus the inorganic form, also appears to be correlated with mineralisable N (Ros et al., 2011). Another important factor is the C/N ratio, as soil C and N cycles are strictly interrelated (e.g. Knicker, 2011). Springob and Kirchmann (2003) showed that mineral N release has a curvilinear relationship with the soil C/N ratio, with the highest values around 10 and a rapid decline when values are around 15.

Among other soil characteristics, soil texture plays a crucial role in influencing SOM mineralisation. Dessureault-Rompré et al. (2010) reported that, except for the labile pool, each pool of mineralisable N increased with higher clay content and lower sand content. This is presumably because of the greater physical protection of organic substrates in clay soils. Soil pH also plays an important role in the soil N cycle: Curtin et al. (1998) showed that the application of limestone materials and the resulting increase in soil pH resulted in increased SOM mineralisation. Specifically, in acidic soils, the intrinsic activity of the microbial community is reduced due to reduced plant production, inhibition of some community members, and increased Al in the soil which induces toxicity (Kemmitt et al., 2006).

The role of microbial activity as driver of SOM mineralisation has been unravelled in several studies. According to the review done by Whalen et al. (2013), more than 10 % of the total soil N content can be potentially mineralised (as PMN) every year by heterotrophic bacteria and fungi. Mineralisation releases N in the form of ammonium, which is converted to nitrate by ammonia oxidisers and nitrifiers. However, although some attempts have been made to integrate microbial abundance and diversity in quantifying C and N cycling in the soil have been made (as reviewed by Louis et al., 2016), the availability of quantitative relationships between the diversity of soil microorganisms and C and N cycles remains a challenge (De Graaff et al., 2015).

Soil life is strongly influenced by environmental factors, and the main environmental factors affecting N mineralisation are soil temperature and moisture (Sierra, 1997). As highlighted by Davidson and Janssens (2006), temperature is a primary factor in the mineralisation process, because the chemical and enzymatic reactions that occur during decomposition depend on temperature. Curtin et al. (2012) found that N mineralisation shows a curvilinear response to increasing temperature in the range of 5 °C to 25 °C, with the highest value around 25 °C. Furthermore, adequate levels of soil moisture facilitate optimal microbial activity, with the highest rates of N mineralisation observed when soil moisture is close to field capacity. Curtin et al. (2012) observed a linear relationship between N mineralisation and soil moisture, with an optimum between 80 % and 100 % of field capacity.

Most investigations on N mineralisation have been conducted in the laboratory under controlled environmental conditions by measuring the stable and labile fractions of organic N and mineral N released by decomposition (Benbi and Richter, 2002). These experiments are useful for studying mechanisms and processes involved in N mineralisation. However, extrapolation of these results to field conditions remains limited by intricate interactions between crops, climatic variables, soil and soil microorganisms. More realistic estimates of N mineralisation can be obtained by combining a set of different measurements under field conditions (Clivot et al., 2017). After comparing many tests (such as CO_2 evolution on rewetting, aerobic incubation, PMN, and extraction with a variety of reagents), Griffin, (2015) concluded that no single test exists that can be used as a reliable estimator of the observed N availability to crops, as measured by the plant uptake. Plant N uptake is the only indicator of agronomic interest in true N availability (Olfs et al., 2005). In a system where external fertiliser inputs are excluded, the N uptake of unfertilised crops can be regarded as a proxy for N mineralisation (De Neve, 2017; Saito and Ishii, 1987; Schepers and Meisinger, 1994). However, the choice of crop is problematic. An ideal crop would be permanent and growing year round, with the theoretical ability to take up mineralised N whenever it occurs, such as grassland. Alternatively, a crop with high N demand and whose growth period is synchronised with the season when most of the mineralisation from SOM occurs, is an acceptable compromise.

Studies that have investigated the impact of environmental and soil indicators on N mineralisation under field conditions have mainly focused on spatial variation, as evidenced by Clivot et al. (2017), Dessureault-Rompré et al. (2010), Gilmour (2021), and Hu et al. (2022). However, it is imperative to recognise the importance of the temporal dynamics of this process, a dimension that is uniquely accessible using long-term data. Long-term field experiments (LTEs) provide valuable information on the changes in SOM mineralisation caused by variations in soil C stocks, environmental and soil indicators, and allow identification of gradual changes in soil fertility, which cannot be evaluated in short-term experiments, as described by Stroud et al. (2022). However, in the context of climate change (Trnka et al., 2011), it is imperative to obtain more precise estimates of the amount of N supplied to crops by SOM mineralisation. Long-term data provides an excellent contribution to the exploration of this issue.

The main goal of this study was to improve the knowledge needed to predict the contribution of N from SOM mineralisation in a provisional nutrient management plan. We hypothesised that the effects of weather conditions and soil properties on this process can be quantified using data from a field experiment, under the assumption that factors that influence SOM mineralisation are the same in both fertilised and unfertilised plots. For this purpose, we used data from an LTE, in which N uptake by unfertilised crops was used as a proxy for N derived from SOM. Specifically, the objectives of this study were: i) to highlight possible differences between cropping systems in serving as proxy of SOM mineralisation; ii) to describe the trends of N mineralisation, meteorological and soil indicators across three decades; and iii) to develop a statistical model to quantify the relative importance and effect of meteorological and soil variables on N mineralised from SOM.

2. Materials and methods

2.1. Site and experiment

The study was conducted on the long-term experimental platform of Tetto Frati (altitude 232 m asl, latitude 44°53’11”, longitude 7°41’09”) of the University of Turin, which has been active since 1992. The trial compared various maize-based cropping systems with different types and amounts of N fertilisation, for a total of 38 treatments, in a randomised block design with 75 m^2-plots and three replicates (Grignani et al., 2007; Zavattaro et al., 2016). Treatments have been repeated on the same plots since 1992. Among all treatments, three unfertilised systems were selected: maize for silage (MS), maize for grain (MG), and maize-Italian ryegrass double cropping (MR). Previous research had shown that maize uptake is well synchronised with mineralisation in this environment (Monaco et al., 2010), but there was no *a priori* reason for excluding one of the two systems with maize as a single crop (MS and MG). We expected that any further mineralisation occurring during autumn and winter could be detected by the winter-cycle Italian ryegrass in the MR system.

No mineral or organic N supply was performed from the beginning of the experiment. In contrast, every year the soil was fertilised with mineral P and K, tilled using a spading machine, then maize was sown in mid-April to mid-May in the MS and MG systems, and at the end of May (after harvesting the Italian ryegrass) in the MR system. The crops were weeded under normal conditions. Sprinkler irrigation of 25–50 mm was performed in summer 0–3 times per year, depending on the weather, to prevent drought stress in the crops. Maize was harvested at the beginning of September in MS, and at the end of September-beginning of October in MG and MR. The maize residue in the MG treatment was chopped and incorporated into the soil through digging in November and December. Italian ryegrass was sown soon after maize harvesting in MR, without any fertiliser supply.

Aboveground crop biomass production was assessed each year in an 18 m^2 sampling area for maize, and 10 m^2 for Italian ryegrass. The production was expressed as oven-dried matter (t $ha^{-1}yr^{-1}$). The crop nutrient uptake (kg N $ha^{-1}yr^{-1}$) was determined from a chemical analysis of N concentration in plant samples at harvest, after pooling the three block replicates. The analysis was performed using an elemental analyser (NA1500 Nitrogen Analyser, Carlo Erba Instruments) (Grignani et al., 2007).

The soil was deep, sandy loam and calcareous, with a pH of 8.1, as reported by Grignani et al. (2007). The groundwater was approximately 6 m deep and did not influence the water and N balance at the site. The climatic conditions at the site were typical of a temperate subcontinental climate, characterised by an average annual precipitation of 741 mm and an average temperature of 12.6°C (Fig. 1). Local measurements of rainfall N concentrations provide estimates of 15 kg $ha^{-1}yr^{-1}$ of N as atmospheric N deposition, ranging from 9 to 22 kg $ha^{-1}yr^{-1}$ (Zavattaro, unpublished data).

This study considered data from 1994 to 2022, for a total of 29 years. Two years before 1994 were excluded to minimise the impact of previous fertilisation. Data from the three replicates were separated.

2.2. Calculation of mineralised N from SOM

The amount of N mineralised from SOM was calculated based on the mass balance of unfertilised plots as the difference between the plant uptake and the sum of N derived from atmospheric deposition and irrigation (Eq.1).

$$Nmineralised = Nuptake - (Ndeposition + Nirrigation) \tag{1}$$

The concentration of N in irrigation water was approximately 10 mg l^{-1}, thus providing the crop with less than 1 kg ha^{-1} yr^{-1} of N (A.R.P.A. Piemonte, 2024). Atmospheric deposition was approximately 15 kg ha^{-1} yr^{-1}, as an average of earlier values of 26 kg ha^{-1} yr^{-1} (Bassanino et al., 2011) and more recent ones of 13 kg ha^{-1} yr^{-1} (Zavattaro, unpublished data). Plant N removal corresponded with the total harvest in the case of MS, while in MG the aboveground plant uptake was used, despite the grain only was harvested. In the case of the MR system, the sum of total harvested maize and Italian ryegrass was considered. N leaching, NH_3 volatilisation and denitrification losses were all considered null, due to the absence of fertilisation, also compared with previous studies at the site (Grignani et al., 2007; Zavattaro et al., 2012).

2.3. Agrometeorological indicators

Daily meteorological data from an electronic station located approximately 200 m from the field trial site were used to compute 15 agrometeorological indicators, as synthetic descriptors of the annual weather patterns (Table 1). All indicators were calculated for a period of one year, starting from the month of harvest and back for the preceding 12 months. The calculated indicators included the mean of daily maximum (T_{max}), minimum (T_{min}), and mean (T_{mean}) air temperatures, mean of daily thermal excursion (Esc) as in Moonen et al. (2002), and growing-degree days (GDDs) on a 0, 5 and 10°C basis (Qian et al., 2010). Indicators regarding rainfall always included the sum of rainfall and irrigation, and were the annual sum of rainfall and irrigation (R.I.; Moonen et al., 2002); the coefficient of variation of rainfall and irrigation (CV R.I.), defined as the ratio of the standard deviation of monthly cumulative precipitation and irrigation to the corresponding mean, expressed as a percentage (Reig-Gracia et al., 2022); and the period of drought (Dry), calculated as the average number of consecutive dry days following the first dry day after a rainy event (Hills and Morgan, 1981), with a threshold of 1.0 mm, thus assuming that any precipitation below this threshold is evaporated (Mathugama and Peiris, 2011). Evapotranspiration was calculated following the Penman-Monteith equation (ET0; Allen et al., 1998), and reported as an annual sum. Additionally, a simple soil water balance was calculated using the sum of rainfall and irrigation minus ET0 (WB) (Moonen et al., 2002). In addition, three aridity indices were calculated: De Martonne Aridity Index (DMI), UNEP Aridity Index (AI), and Emberger aridity index (EAI). DMI was calculated as the ratio between cumulative precipitation and irrigation, and the annual average temperature increased by 10°C (de Martonne, 1926). AI was calculated as the ratio of cumulative precipitation and irrigation, and cumulative evapotranspiration (Huang et al., 2016). EAI was

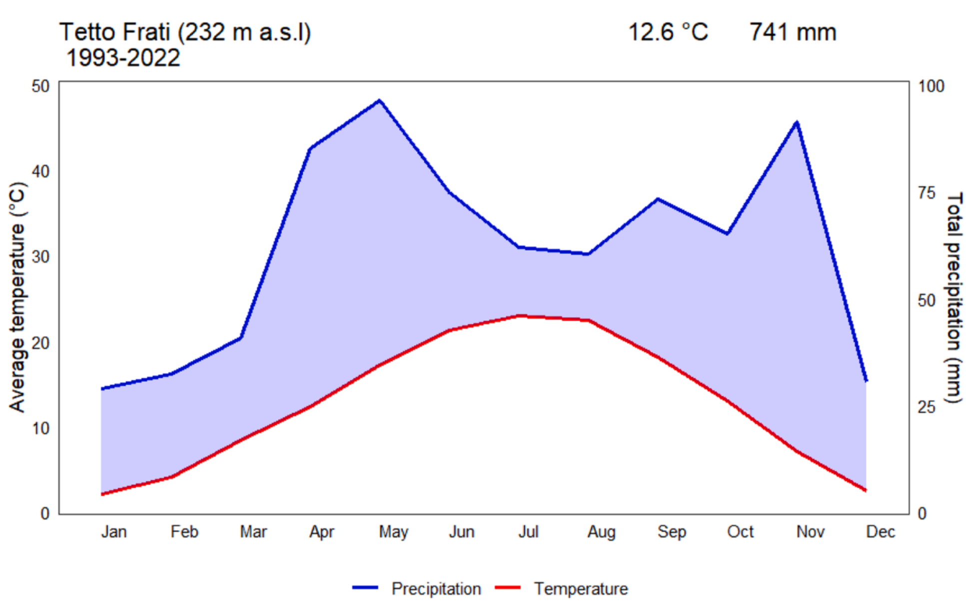

Fig. 1. Walter and Lieth climate diagram at the Tetto Frati LTE in the time span of this study. Precipitation does not include irrigation, in contrast to the agrometeorological indicators of Table 1.

Table 1
Agrometeorological indicators calculated at the LTE.

Name	Abbreviation	Unit	Formula	Reference
Maximum air temperature	T_{max}	°C	$T_{max}=\frac{\sum_{i=1}^{n} Tmax_i}{n}$	Moonen et al. (2002)
Minimum air temperature	T_{min}	°C	$T_{min}=\frac{\sum_{i=1}^{n} Tmin_i}{n}$	Moonen et al. (2002)
Mean air temperature	T_{mean}	°C	$T_{mean}=\frac{\sum_{i=1}^{n} Tmean_i}{n}$	Moonen et al. (2002)
Growing-degree days with a 0°C base	GDD.0	°C	$GDD.0=\sum_{i=1}^{n} \frac{Tmax_i + Tmin_i}{2}$; $GDD.0=0$, if $\frac{Tmax_i + Tmin_i}{2} < 0°C$	Qian et al. (2010)
Growing-degree days with a 5°C base	GDD.5	°C	$GDD.5=\sum_{i=1}^{n} (\frac{Tmax_i + Tmin_i}{2} - 5°C)$; $GDD.5=0$, if $\frac{Tmax_i + Tmin_i}{2} < 5°C$	Qian et al. (2010)
Growing-degree days with a 10°C base	GDD.10	°C	$GDD.10=\sum_{i=1}^{n} (\frac{Tmax_i + Tmin_i}{2} - 10°C)$; $GDD.10=0$, if $\frac{Tmax_i + Tmin_i}{2} < 10°C$	Qian et al. (2010)
Mean of daily thermal excursion	Esc	°C	$Esc=\frac{\sum_{i=1}^{n} (Tmax_i - Tmin_i)}{n}$	Moonen et al. (2002)
Total rainfall and irrigation	R.I.	mm	$R.I.=\sum_{i=1}^{n} (R_i + I_i)$	Moonen et al. (2002)
Coefficient of variation of R.I.	CV R.I.	%	$CV\ R.I.=\frac{\sigma_{R.I.}}{\mu_{R.I.}}$	Reig-Gracia et al. (2022)
Average period of drought	Dry	Number of days	$Dry=\frac{\sum_{i=1}^{n} (days\ with R.I. < 1\ mm)}{n}$	Mathugama and Peiris (2011)
Reference evapotranspiration	ET0	mm	$ET0=\sum_{i=1}^{n} ET0_i$	Allen et al. (1998)
Water balance	WB	mm	$WB = R.I. - ET0$	Moonen et al. (2002)
De Martonne Aridity Index	DMI	-	$DMI=\frac{R.I.}{Tmean + 10°C}$	de Martonne (1926)
UNEP Aridity Index	AI	-	$AI=\frac{R.I.}{ET0}$	Huang et al. (2016)
Emberger aridity index	EAI	-	$EAI=\frac{R.I.}{T_{hottest\_month}^2 - T_{coldest\_month}^2}$	Emberger (1930)

i = days between maize harvest month and the preceding 12 months

calculated as the ratio between cumulative precipitation and irrigation, and the difference between squared average temperature of the hottest month and squared average temperature of the coldest month, both expressed in Kelvin degrees (Emberger, 1930).

In general, during the observation period, the climate at the site was humid throughout the year (Fig. 1).

2.4. Soil indicators

From 1999 to 2018, soil organic C (SOC) and total N content (TN) were determined every three years in each plot, for a total of seven sampling times in the analysed time frame. Soil samples were randomly collected in March at a depth of 0–30 cm by pooling three subsamples per plot. SOC and TN were analysed using the Carlo-Erba Elemental protein NA2100 analyser after subtracting inorganic C, which was determined using a Dietrich-Fruhling calcimeter. The first two soil indicators were the concentrations of SOC and TN expressed in g kg^{-1}. The third indicator was the C/N ratio (C/N), calculated from the measured samples at the plot scale. Further details have been reported by Grignani et al. (2007) and Zavattaro et al. (2016).

As the soil indicators were discontinuous in time, a linear interpolation and extrapolation of the measured values was performed to obtain a value every year. The assumption made for interpolation is that long-term soil properties can be described as trends, if the sampling frequency is adequate (Capriel, 2013). The cropping system and the three replicates were kept separated.

2.5. Data analysis

Data analysis was conducted using R version 4.2.3 for the Windows operating system (R Core Team, 2023).

2.5.1. Mineralised N in the different cropping systems

To test for differences in mineralised N from SOM among the three cropping systems over the entire timespan, a nonparametric test was chosen because of the violation of the normality assumption in mineralised N data. For this purpose, the Kruskal-Wallis Test was performed using the *kruskal_test* function of *rstatix* package (Kassambara, 2023). Subsequently, the pairwise Wilcoxon Rank Sum Test was performed using *pairwise.wilcox.test* function of the basic *stats* package.

2.5.2. Trend of mineralised N, agrometeorological and soil indicators over time

A linear regression model was used to describe the trend of mineralised N as a function of agrometeorological and soil indicators from 1994 to 2022. The *lm* function of the *stats* package was used to perform linear modelling.

2.5.3. Effect of soil and agrometeorological indicators on mineralised N

A linear mixed model (LMM) was used to evaluate the effect and relative importance of agrometeorological and soil indicators on mineralised N. The first step was the selection of uncorrelated variables that could be used in the model (Zuur and Ieno, 2016). A correlation matrix between all agrometeorological and soil indicators was generated using Spearman's correlation coefficient ($|\rho|<0.6$) in the *cor* function of the *stats* package. Spearman's correlation was chosen to evaluate monotonic relationships between variables, whether linear or not. Next, non-correlated independent variables were selected using the *findCorrelation* function in the *caret* package (Kuhn, 2008).

The variables that met the above criteria were T_{min}, Esc, Dry, R.I, SOC, and C/N. They were subsequently standardised (z-scores) to assess the relative weight using the *scale* function from the basic *base* package. Then, standardised variables were used as explanatory variables of mineralised N in a full factorial LMM using the *lmer* function from the *lme4* package (Bates et al., 2015). The LMM equation is (Eq.2):

$$Y_i = (\alpha_i\,|Year) + (\beta_i\,|Treatment) + \gamma_1 * T_{min} + \gamma_2 * Esc + \gamma_3 * Dry + \gamma_4 * R.I. + \gamma_5 * SOC + \gamma_6 * C/N + \varepsilon_i \quad (2)$$

where Y_i was mineralised N where the three cropping systems and the three replicates were kept separated. "Year" and "Treatment" were considered as random crossed effects, whereas "T_{min}", "Esc", "Dry", "R.I.", "SOC" and "C/N" were considered as fixed effects. The coefficients denoted as "α, β, γ" correspond to the respective coefficients associated with each variable, while "ε" denotes the error term.

In the LMM model, a stepwise variable selection procedure was performed in which only statistically significant variables were preserved. The *step* function of the *lmerTest* package (Kuznetsova et al., 2017) was used for this purpose. The selected variables were "R.I.", "SOC" and "C/N".

The *performance* function from the *performance* package (Lüdecke et al., 2021) was used to assess the coefficient of determination (R^2) and root mean square error (RMSE). In addition, the *check_model* fuction was used to visually verify the model assumptions. The *ggplot* function from the *ggplot2* package (Wickham, 2016) was used to create all figures.

3. Results

3.1. N mineralised in the three cropping systems

Considering all 29 years, N taken up by the crop ranged from 27 to 201 kg ha^{-1}yr^{-1}, with a mean of 96 kg ha^{-1}yr^{-1} and a median of 90 kg ha^{-1}yr^{-1} (Fig. 2).

The N mineralised from SOM, calculated as in eq. [1] by subtracting 16 kg ha^{-1} of N from the plant uptake, was different in the three cropping systems. In particular, MG had the highest median of 95 kg ha^{-1}yr^{-1}, and MR had the lowest median of 86 kg ha^{-1}yr^{-1}, with MS showing intermediate values, with a median of 91 kg ha^{-1}yr^{-1} (Fig. 2).

3.2. Trend of mineralised N, agrometeorological and soil indicators over time

The N mineralised from SOM had high annual variability, as shown in Fig. 3. A decreasing trend over the years was observed when the three systems were pooled together, from an average of 123 kg N ha^{-1} in 1994–89 kg N ha^{-1} in 2022. This trend indicated a decrease of approximately 1 kg N ha^{-1} per year (R^2 =0.07, p-value < 0.001).

As shown in Fig. 4, the temporal evolution of agrometeorological indicators across the three decades also revealed a clear trend of increasing temperatures (by an average of 0.04°C every year) and decreasing water balance (by an average of 8 mm per year). However, among the aridity indices, only AI showed a significant decreasing trend of 1 % every year, passing from values previously greater than 1 during the 1990s (indicating a condition in which rainfall and irrigation exceeded evapotranspiration), to values smaller than 1 since 2016 (indicating that evapotranspiration exceeded rainfall and irrigation), except for 2020 (AI= 1.05).

The trends across the 29-year span of SOC and TN concentrations and C/N ratios of all cropping systems are shown in Fig. 5. Although the slopes were different among crop systems and replicates, a general decreasing trend for both SOC and TN was observed. The average SOC loss was 0.061, 0.064 and 0.057 g kg^{-1}yr^{-1} in MG, MS and MR systems, respectively. The average TN loss was 0.010 g kg^{-1}yr^{-1} in all the systems. Consequently, the C/N increased on average by 0.018, 0.017, and 0.024 each year in the MG, MS, and MR systems, respectively.

3.3. Effect of soil and agrometeorological indicators on mineralised N

The results of the stepwise LMM for predicting mineralised N are presented in Table 2. SOC and R.I. had a positive effect and equal weight on mineralised N, whereas C/N had a negative effect and a lower weight on the process. The conditional R^2, representing the variability explained by the fixed factors, reached 19 % of the observed variability. However, when including year and cropping system as random factors, the marginal R^2 reached 52 % of the observed variability. The RMSE of the model was 21.8 kg N ha^{-1}.

As illustrated in Fig. 6, which reports observed versus predicted values, the stepwise LMM showed a tendency to overestimate mineralised N values below the threshold of 96 kg N ha^{-1}, while it tended to underestimate values above it.

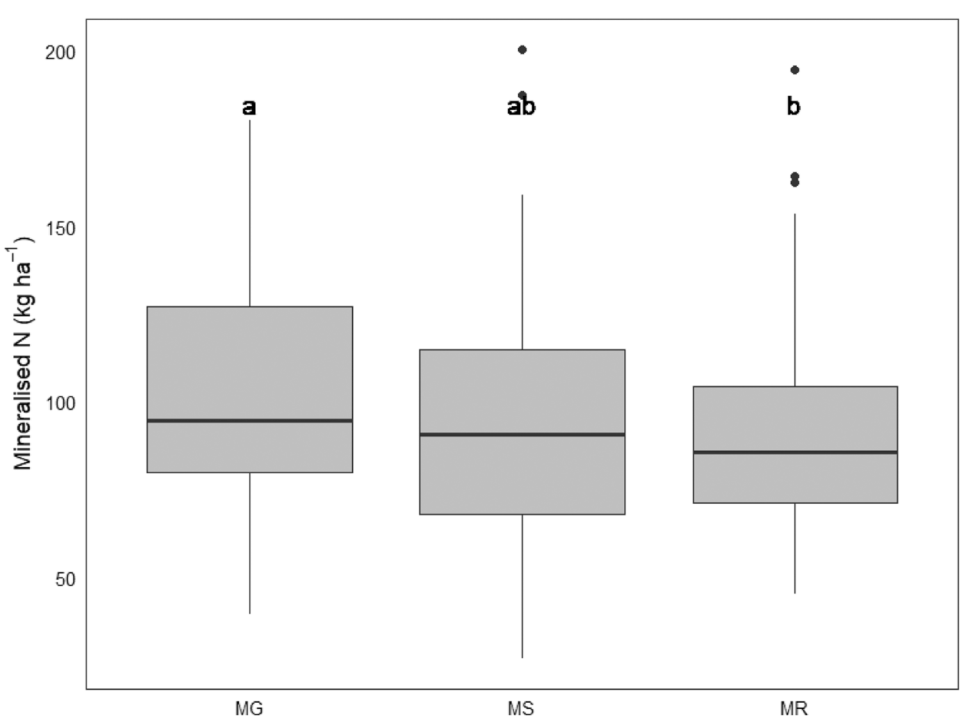

Fig. 2. Boxplot of mineralised N in each system. Letters indicate significant differences at Wilcoxon rank sum test. MG= maize for grain, MS= maize for silage, MR= maize-It. ryegrass double cropping.

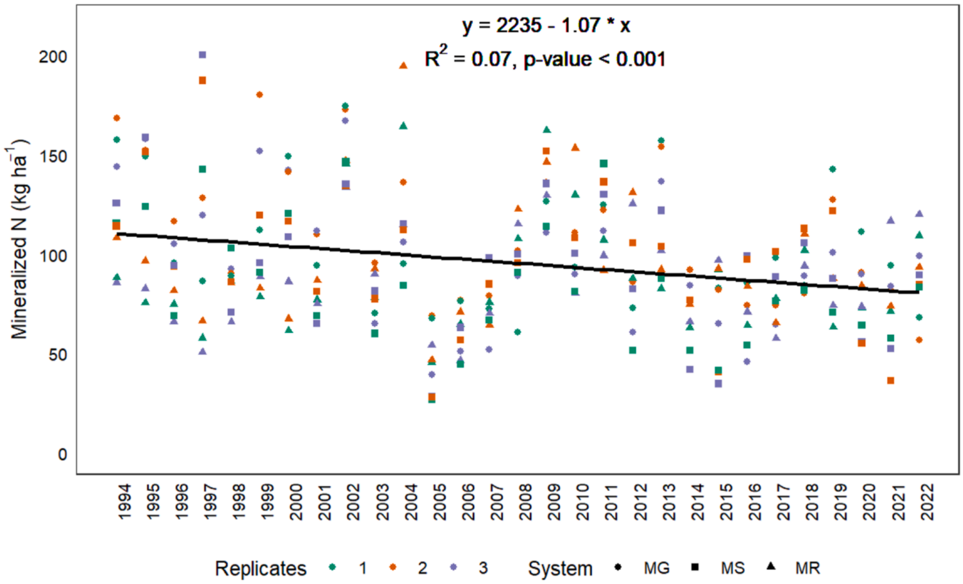

Fig. 3. Trend of N mineralised each year from SOM in the three systems. MG= maize for grain, MS= maize for silage, MR= maize-It. ryegrass double cropping.

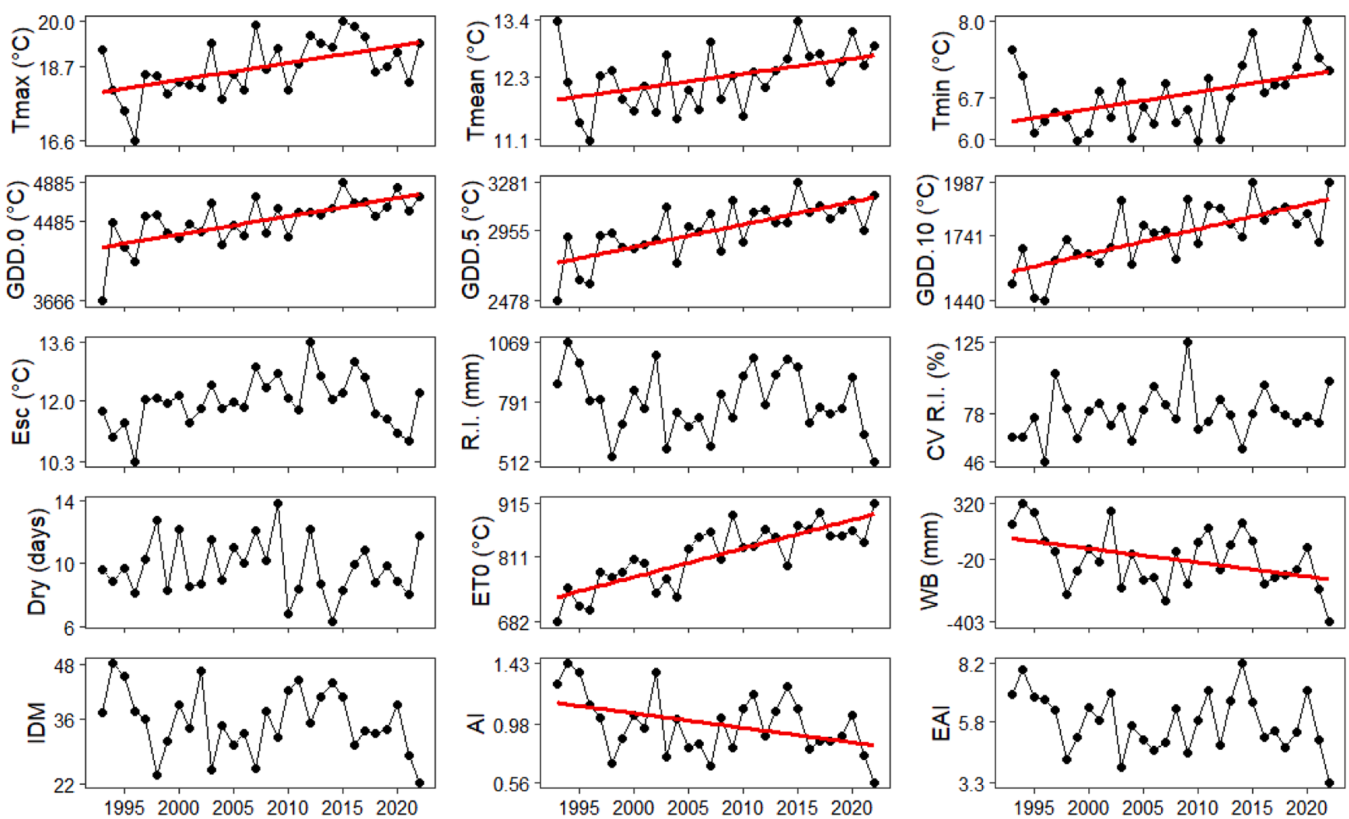

Fig. 4. Trends of agrometeorological indicators (max, mean, min) across the 29-year span of the study (1994–2022). When statistically significant ($p < 0.05$), linear regressions are shown.

4. Discussion

In the absence of N fixation, to obtain the N needed for growth, unfertilised crops can rely only on the mineralisation of SOM and other minor sources such as atmospheric N deposition, irrigation, and groundwater contributions. These minor sources, whose importance depends on the site-specific conditions, at Tetto Frati provided about 16 kg ha^{-1}yr^{-1} of mineral N. If we exclude this extra input, the average amount of N mineralised from SOM at Tetto Frati was 96 kg ha^{-1}yr^{-1} (Fig. 2), which is about 1.9 % of soil TN content (if we consider a depth of 30 cm and a soil bulk density of 1.3 t m^{-3}). This result is consistent with that reported by Bertora et al. (2009), who calculated an annual mineralisation rate of 1.6 % in MS plots using an inverse modelling approach. However, a large variability was observed around the mean value of N mineralised by SOM (Fig. 3).

The hypothesis of this study was that temporal variations of agro-

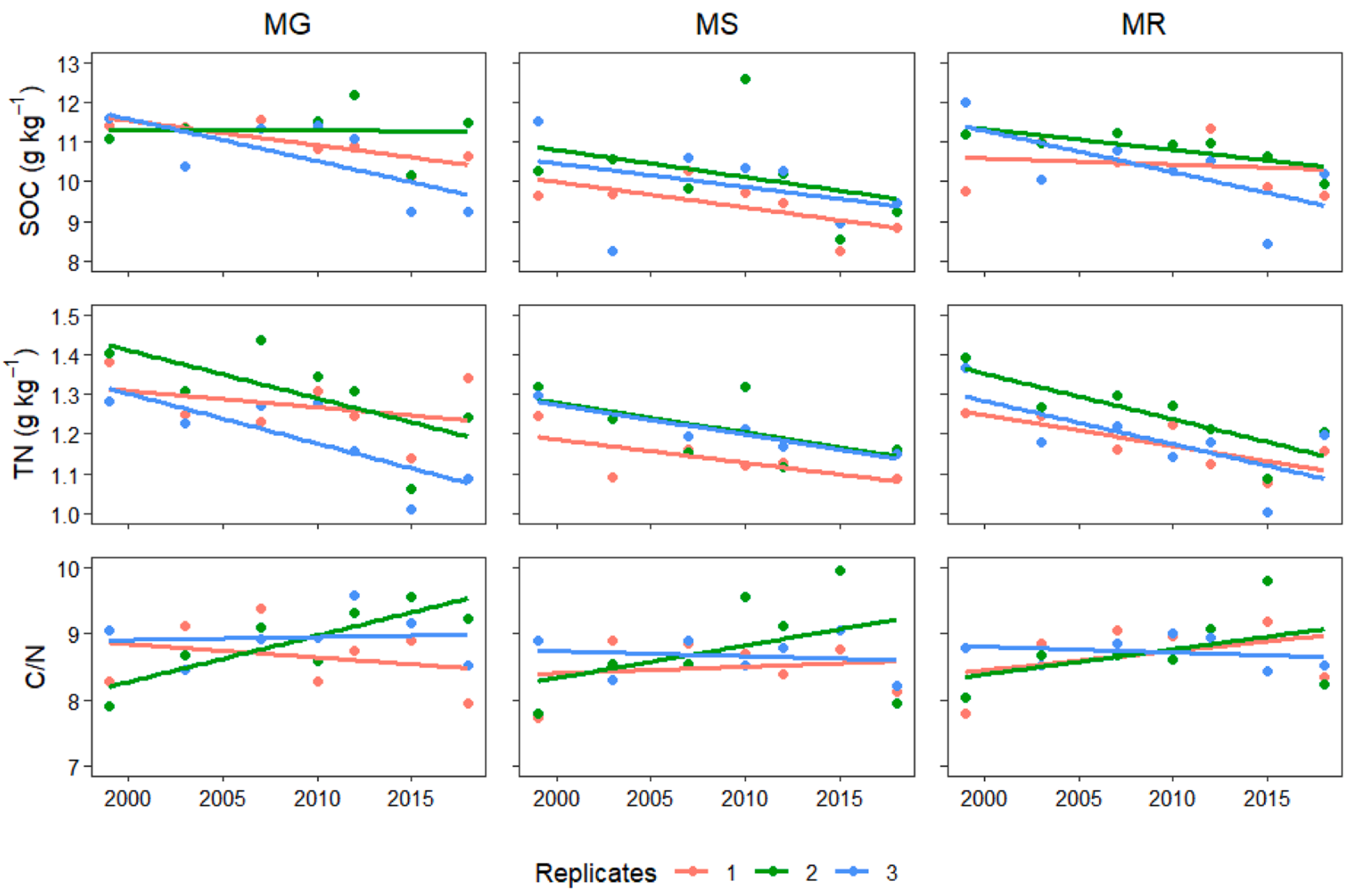

Fig. 5. Trends of soil indicators (points are measured values, and lines are linear interpolations in time). MG= maize for grain, MS= maize for silage, MR= maize-It. ryegrass double cropping, SOC= soil organic C, TN= total N content, C/N = C:N ratio.

Table 2
Result of the LMM stepwise model using standardised variables.

Fixed factors						Random factors			Performance	
	Estimate	S.E.	df	t	Pr(>|t|)		Variance	S.D.	AIC	2435
Intercept	95.7	4.3	15	26.1	< 0.001	Year	355.8	18.8	R^2 (cond.)	0.522
R.I	9.4	3.8	29	2.4	0.020	Treatment	12.0	3.5	R^2 (marg.)	0.190
SOC	9.3	2.3	29	3.9	< 0.001	Residual	528.6	23.0	RMSE	21.80
C/N	−3.7	1.8	258	−2.1	0.038					

R.I.= rainfall and irrigation, SOC=soil organic C, C/N = soil C:N ratio

meteorological indicators, together with SOM amount and characteristics, could explain the observed annual variations of N mineralised from SOM. We observed three main sources of variation: a) the cropping system used for the estimation, b) a long-term decreasing trend, and c) interannual fluctuation. We linked the latter two factors to variations in the soil and meteorological conditions.

The amount of N mineralised from SOM varied depending on the cropping system used. Despite criticism of the use of unfertilised crops as a proxy for mineralised N, especially with regard to the omission of N losses to the environment and the uptake of N in the unharvested parts of crops, such as roots (Jarvis et al., 1996), plants remain the only true indicator for applied studies on fertilisation planning (Kindred et al., 2012). On average, our study found that 2.0 %, 1.9 %, and 1.8 % of the total N in the 0–30 cm layer was mineralised for the MG, MS, and MR systems, respectively. The statistically significant differences between maize for grain and maize-Italian ryegrass double-cropping systems (Fig. 2) can be attributed to different SOM types, whereas the return of crop residues in MG did not represent an extra input of N. Dămătîrcă et al. (2023) showed that long-term incorporation of maize residues in the MG system compared to the MS system at Tetto Frati resulted in increased N concentration in the associated stable mineral fraction (MAOM), which contributed up to 97 % of TN in the top 30 cm of soil. This means that maize residues, which are rapidly decomposed at this site (Bertora et al., 2009; Pulina et al., 2022), do not contribute to maize nutrition, but rather remain in the soil as stable SOM fractions. The MR had the longest soil cover among the three systems studied (11 months per year). Nevertheless, the N uptake of this system as a sum of both crops was not as large as when only maize was cultivated. Our interpretation is that maize as a single crop is particularly synchronised with SOM mineralisation in the studied environment and absorbs mineralised N to the greatest extent (Monaco et al., 2010). The synchronisation of plant uptake and N mineralisation should be intended as an active stimulation of plants for the decomposition of MAOM, in line with the ideas of Fontaine et al. (2024) and on recent paradigms that reverse the role of plants in N fluxes from passive users of microbial activity outcomes to active modifiers of soil processes through microbial stimulation (e.g. Daly et al., 2021). Italian ryegrass termination, tillage, and maize sowing may have temporarily interrupted the plant regulating

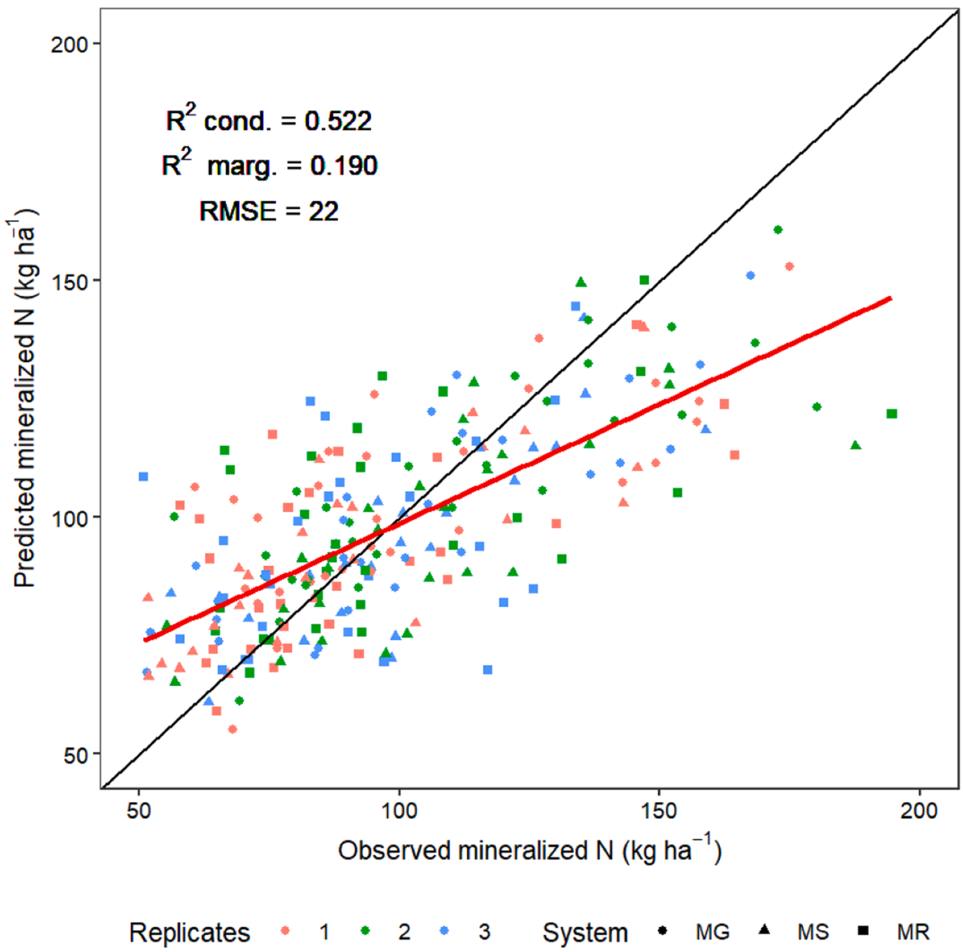

Fig. 6. Observed versus predicted mineralised N. The red line represents the model fit. MG= maize for grain, MS= maize for silage, MR= maize-It. ryegrass double cropping.

system of SOM decomposition. Based on the similarity of results among the three cropping systems studied here, all of them seem adequate to estimate the amount of N mineralised from SOM. Consequently, they were kept together in the formulation of the statistical model by considering the cropping system as a random factor.

The second source of variation observed in N mineralised from SOM was a gradual decline over the 29 years of observation (Fig. 3). The decline was of about 1 kg ha^{-1}yr^{-1}, i.e. 0.02 % of the soil TN content in the 0–30 cm layer. If we express N mineralised in relation to the soil TN content, it emerged that its overall variability was quite large (0.6–3.4 %), but no temporal trend was observed. Therefore, the temporal decline in mineralised N expressed as an absolute value can be attributed to the observed decline in SOM content, and the interannual variability was mainly due to meteorological conditions. Consistently, the LMM approach used in this study showed that the variability of mineralised N was explained by three fixed factors (Table 2), and two of them, SOC and C/N, regarded the availability and type of substrate.

The reduction in SOC and TN concentrations was in line with observations from in other LTEs in Italy (Lugato et al., 2007; Mazzoncini et al., 2016). For all cropping systems, there was a greater loss of TN than of SOC (Fig. 5), thus modifying the soil C:N ratio over time. As Mazzoncini et al. (2016); Osterholz et al. (2017); White et al. (2021) reported, the soil organic matter content (expressed as SOC or TN content) is the first driver of mineralised N; in other words, in the presence of increased substrate availability, there is an increase in mineralisation. Our study confirmed this, as SOC concentration emerged as the second most relevant factor explaining N mineralisation from SOM. In addition, according to the LMM model, a decrease in mineralised N was observed with an increase in the C:N ratio, consistent with previous studies (e.g. van der Sloot et al., 2022).

The increase in air temperatures (annual maximum, minimum, and mean) observed at the Tetto Frati LTE across the 29 observation years (Fig. 4) – approximately + 0.04°C per year – is probably linked to the general increase in temperatures observed in the area (Fioravanti et al., 2016). According to laboratory studies, the effect of temperature on mineralisation is positive, and it is the main variable affecting the process (Guntiñas et al., 2012). In field conditions, soils experience a diurnal excursion and seasonal variations that cannot be recognised in indicators calculated on an annual scale. The increase in air temperature was so prominent in the observed time frame that maximum daylight temperatures likely hampered the full potential of microbial activity and could therefore be a second cause for the observed decrease in its entity over the years. Although we cannot support this hypothesis with our data, the literature provides examples of a decrease in mineralisation under the specific climate conditions of our study (Elrys et al., 2021). Soil temperatures at a depth of 12 cm, which were measured at Tetto Frati for some years (data not shown), were correlated with air temperatures and were even 1.1°C higher during the crop growing season. Under these conditions, microbial activity may have decreased in the summer.

Another factor which could have contributed to the decrease in microbial activity is the diminishing trend in soil available water. The Water Balance (WB) indicator showed a significant trend across the observed time span (Fig. 4). This trend was caused by an increase in ET0 rather than a decrease in rainfall, while rainfall showed no clear trend over time. In addition to the WB, the AI indicator also showed a decrease in soil water availability between 1994 and 2022.

The third observed source of variability was the interannual fluctuations. We attributed these variations mainly to water availability, which emerged, expressed as the R.I indicator, as the main factor influencing N mineralised from SOM in the LMM model (Table 2). According to the literature, a positive effect of water availability on N mineralisation is expected because of the positive correlation between soil moisture, microbial activity, and crop production (Bocchiola et al., 2013; Whalen et al., 2013). As recalled by Whalen et al. (2013), soil organisms depend on soil moisture for survival, and consequently, biochemical reactions such as N mineralisation and nitrification require a certain amount of soil water content.

The LMM model revealed that soil and meteorological indicators explained 19 % of the annual variations in mineralised N (Table 2). When other influential factors, such as the cropping system and interannual variability, were included as random factors, the explanatory power of the model increased to 52 %. Consequently, there are other factors influencing mineralised N from SOM under field conditions that remain unexplained. The LMM showed a tendency to overestimate mineralised N values below the threshold of 96 kg N ha^{-1}, while it tended to underestimate the values above it (Fig. 6). Adopting a model always reduces the complexity of the dependent variable over time, and thus the explained variability, which leads to a levelling of predicted values and consequent overestimates and underestimates. However, the RMSE of the predictions was 21 kg $ha^{-1}yr^{-1}$ only. Such an error is acceptable when this estimate is used in a provisional Nutrient Management Plan based on mass balance (Bassanino et al., 2011; Bechini and Castoldi, 2006).

The approach used in this study allowed for the identification of the main causes of variations in the amount of N that is made available to crops due to SOM mineralisation. However, there are some limitations, in particular the use of data from only one site, limited availability of soil analyses (7 years out of 29), and only moderate model performance. The data analysed came from the Tetto Frati LTE, which allowed us to focus on a long time series of crop and weather data, but with limited variability in soil properties and a limited number of soil analyses. This platform was not designed to study SOM dynamics; therefore, the data availability was incomplete. The adopted LMM has the advantage of considering many possible types of variables, but it uses linear relationships, which are not always the best approach to describe biological processes. On the other hand, because of the high correlation between explanatory variabilities, the selection process eliminated some of them that we would expect to find in the final model (e.g. temperature). This may justify the low variability explained by fixed factors. In fact, by using data from different sites, thus increasing soil and climate variability and considering different crops, model performance is expected to increase.

Despite their limitations, in the context of climate change, field studies are needed to quantify the extent to which water scarcity and rising temperatures limit not only crop growth, but also microbial regulation of N supply (Greaver et al., 2016). The findings of our study are alarming in a future scenario in which temperatures are expected to increase, and rainfall is expected to decrease and change in frequency and intensity. Even under the premise of constant rainfall, an increase in extreme rainfall frequency will lead to a decrease in the availability of rainfall to plants and biological soil processes (Tramblay and Somot, 2018). Bocchiola et al., (2013) showed that maize cropping systems of the same area as our study site will require more irrigation to ensure profitable yields in the future. Consequently, a future shortage in rainfall might not only affect crop productivity, water resources, and the agricultural water footprint, but also the ability of the soil to supply N to plants, thus requiring additional fertiliser use; instead, a reduction is claimed by environmental concerns.

5. Conclusions

This investigation was performed over almost three decades over a Long-Term Experiment and showed that the water supply, soil organic carbon, and C:N ratio affected the amount of N mineralised from SOM. With an average of 96 kg ha^{-1} yr^{-1}, and 1.9 % of soil TN content, SOM mineralisation showed relevant variability in time, of which only 19 % could be explained by meteorological and soil indicators. However, the model prediction accuracy is sufficient for use in a provisional nutrient management plan, under the assumption that factors influencing SOM mineralisation are the same regardless of the fertilisation regime.

In a future climate scenario, in which temperatures are expected to increase and rainfall patterns are expected to change, N mineralisation could therefore decrease. This could result in the additional use of fertilisers, as opposed to environmental regulations that require their reduction.

The framework of this study was limited to data from a single site and to the use of a single crop as a reference. Future research efforts should extend this investigation by examining the impact of soil and meteorological factors on N mineralisation under different soil and climate conditions and with multiple crops.

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CRediT authorship contribution statement

Octavian P. Chiriac: Writing – original draft, Writing – review & editing, Methodology, Formal analysis. **Marco Pittarello:** Supervision, Writing – review & editing, Methodology. **Barbara Moretti:** Writing – review & editing, Data curation. **Laura Zavattaro:** Supervision, Writing – review & editing, Methodology, Funding acquisition, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Paperpal in order to improve language. After using this tool/service, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

Declaration of Competing Interest

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

Data availability

I have shared the link to database as SI.

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