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Effects of moisture and temperature on net soil nitrogen mineralization: A laboratory study

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ABSTRACT

Climate change will lead to changes in soil moisture and temperature, thereby affecting organic matter mineralization and the cycling of biophilic elements such as nitrogen. However, very few studies have considered how the sensitivity of the rate of net nitrogen mineralization to temperature and/or moisture content may be modified by changes in these parameters. To investigate how changes in temperature and moisture content affect net nitrogen mineralization (as regards both the mineralization rate and the sensitivity of the mineralization rate to changes in temperature and moisture content), a laboratory experiment was carried out in which three soils under different types of use (Forest, Grassland, Cropland) were incubated for 42 days under different moisture conditions (between 40 and 100% field capacity) and temperatures (between 10 and 35 °C); total inorganic nitrogen levels were determined at different times throughout the experiment. The rate of mineralization was determined at each temperature and moisture level considered, by use of the mono-compartmental model developed by Stanford and Smith (1972). For all soils, changes in the rate of mineralization with temperature followed the pattern described by the Q_{10} model, while the models used to determine the effect of moisture content on the net rate of mineralization (linear, semilogarithmic, partial parabolic and complete parabolic) were only verified for the Forest soil. In general, the sensitivity to temperature was maximal at 25 °C, and the optimal moisture content for nitrogen mineralization was between 80% and 100% of field capacity. A relatively simple model that included the temperature–moisture–time interaction was also tested. This model provided a significant fit for the three soils under study, in contrast with the other models tested. In any case, further studies are necessary in order to address the extent to which changes in the quality of organic matter, caused by land use, affect any modifications to soil nitrogen that may be generated by climate change.

1. Introduction

Climate is changing on a global scale and affecting soil temperature and moisture regimes [1]. Climate change will therefore also affect all edaphic processes that depend on soil temperature and moisture, including soil organic matter mineralization [2,3]. There has been great interest in the possible effects of climate change on soil carbon cycling and on CO_2 emissions [4,5]. There are two main reasons for this interest. Firstly, as CO_2 is one of the main greenhouse gases (i.e. it is capable of affecting climate change), it is essential to understand the feed-back mechanisms between climate change and CO_2 emissions. Secondly, measurement of CO_2 emissions is a straightforward, rapid method of determining the impact of climate change on edaphic metabolism [4].

However, carbon is not the only element affected by climate change, and elements such as nitrogen, phosphorus and sulphur, typically associated with organisms, are also affected. All of these are essential elements and are therefore extremely important for the correct functioning of ecosystems. In the case of nitrogen, most bioavailable forms of this element in natural soils are produced by mineralization of organic matter via depolymerization of large organic polymers, so that the productivity of many ecosystems will depend directly on the availability of nitrogen derived from the decomposition of organic remains. It is therefore reasonable to assume that any modification that affects the rate of decomposition of organic matter will also affect the availability of nitrogen, and therefore will have important repercussions for ecosystem functioning [6].

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Many studies involving mineralization of organic nitrogen compounds in soil have been undertaken since the early studies in 1972 by Stanford and Smith, as reported by Dessurealt-Rompré et al. [7]. Stanford and Smith [8] demonstrated that net nitrogen mineralization followed first order kinetics and that the effect of temperature on nitrogen mineralization followed the Arrhenius law, with the rate doubling for each 10 °C increase in temperature. Various authors have recently discussed the effects of temperature and/or moisture content on the net mineralization process, as well as how soil moisture should be expressed (percentage of pore filled, water potential, etc.) and how the different mathematical models used to study such relationships should be interpreted [2, 7, 9, 10, 11, amongst others]. Most of these studies have focused on the rate of nitrogen mineralization, with the aim of estimating the amount of nitrogen that is available to plants. In other words these studies had an agronomic focus. Nevertheless, very few studies about soil nitrogen mineralization consider the sensitivity of the mineralization rate to changes in temperature and moisture with the aim of determining the direct impact of climatic parameters on the nitrogen cycle [7]. This is an important omission as more accurate information about the sensitivity of mineralization constants and how they vary in relation to climatic parameters would enable better description of the response of soil organic matter to climate change. Furthermore, there is even less information about the combined effects of these two factors (temperature and soil moisture) on net nitrogen mineralization processes [11–13], despite evidence that climate change will affect both components of the climate simultaneously, as indicated above. In fact, Craine and Gelderman [14] indicated their surprise about the scarcity of information as to how soil moisture affects the temperature sensitivity of soil organic matter decomposition and whether or not any such relationships are consistent across different soils.

At present the climate in Galicia (NW Spain) is humid temperate and, because of its geographical position (north of 40° N), the region is expected to undergo significant changes [1]. It is therefore important to predict the response of soils to such changes, and to establish which types of soils (in terms of management regimes) will be most affected. Recent laboratory and field studies in Galicia have addressed the issue of how CO_2 emissions in soils will be affected by climate change [15]. The thermodynamic properties of several edaphic enzymes (both hydrolases and oxidoreductases) have also been characterized in order to establish how decomposition processes mediated by these enzymes would be affected by changes in soil temperature [16]. However, to date the effects of climate change on nitrogen mineralization have not been analyzed in Galician soils. Therefore, and given the general lack of knowledge about the influence of climate changes on net nitrogen mineralization, the objectives of the present study were: a) to investigate how net nitrogen mineralization is modified in response to changes in temperature or moisture, b) to determine the extent to which land use modifies the sensitivity of net nitrogen mineralization to changes in soil temperature and moisture, and c) to investigate the extent to which climate change will affect nitrogen availability in Galician soils.

2. Materials and methods

2.1. Soils, soil sampling and soil preparation

Three soils (Forest, Grassland and Cropland) were selected as representative of Galician soils under different types of land use. The forest soil (Forest) is an *Umbrisol* under Atlantic oak, which is the *climax* vegetation in Galicia. The grassland soil (Grassland) is also an *Umbrisol* but subjected to intensive grassland use and represents intensively managed grassland soils, derived more than 40 years ago from former forest soils, and fertilized intensively every year, mainly with cattle slurry (200–250 kg nitrogen ha^{-1} $year^{-1}$). Finally, the cropland soil (Cropland) is an *Umbrisol* partially degraded by intensive use, which represents the characteristic type of agricultural soil found close to houses in rural areas of Galicia, and cultivated throughout the year by crop rotation and intense application of both organic (manure) and inorganic (NPK) fertilizers (approximately 150 kg of nitrogen ha^{-1} $year^{-1}$). The location and the main physical and chemical characteristics of each of the soils are shown in Table 1.

The upper 10 cm of each of the soils (A horizons) were sampled. Sampling was carried out after a period of more than 20 days without precipitation (so that the soils were as dry as possible and could be watered to produce the different moisture contents required), and at least one month since the last agricultural treatments in the Grassland and Cropland soils (to minimize the possibility that any responses were not affected by agricultural activities). In the laboratory, the soils were sieved (<4 mm), the fine roots were removed (manually) and the soils were homogenized. The moisture content was determined by gravimetry, in an aliquot of moist soil dried at 105 °C for 24 h. The soils were then placed in plastic bags and stored at 4 °C until the start of the incubation experiment. An aliquot of each soil was used to determine the water retained at −33 kP pressure (field capacity), with a tension plate apparatus [17]. Another aliquot of each soil was air-dried and used for the general analyses outlined further below.

2.2. Incubation procedure and laboratory measurements

The effect of temperature and moisture on soil organic nitrogen mineralization was investigated in a laboratory incubation experiment of 42 days duration. Aliquots of each of the soils (equivalent to 500 g of oven dry soil) were wetted with distilled water to produce different moisture levels. In the Forest and Grassland soils, four moisture levels (40, 60, 80 and 100% field capacity) were considered (corresponding to 34, 51, 68 and 85 g H_2O 100 g^{-1} soil for the Forest soil, and 24, 36, 48 and 60 g H_2O 100 g^{-1} soil for the Grassland soil).

Table 1
Main site characteristics and physicochemical properties of the soils used in the study. Estimated values of potentially mineralizable nitrogen (N_o), expressed as absolute values (mg N kg^{-1} soil) as well as per unit of carbon (mg N g^{-1} total C) and total nitrogen (mg N g^{-1} total N). Numbers in the same row followed by the same letter are not significantly different ($P < 0.001$).

Soil	Forest	Grassland	Cropland
Site characteristics			
Main plant species	*Quercus robur* L.	*Lolium multiflorum* Lam	*Zea mays* L.
Soil parent material	schists	granodiorites	granodiorites
Longitude	8° 21′ 30″ W	8° 42′ 30″ W	8° 42′ 35″ W
Latitude	42°36′ 58″ N	42° 59′ 00″ N	42° 59′ 10″ N
Elevation (m.a.s.l.)	645	320	315
Soil type (FAO)	*Umbrisol*	*Umbrisol*	*Umbrisol*
Physicochemical soil properties			
Soil pH (in water)	4.81	5.43	5.01
Total C (%)	8.82	8.26	5.22
Total N (%)	0.88	0.70	0.47
C/N	10	12	11
Clay content (%)	24	17	20
Soil texture	sandy loam	sandy loam	sandy loam
Field capacity (g H_2O g^{-1} soil)	0.85	0.60	0.42
Potentially mineralizable nitrogen (N_o)			
mg N kg^{-1} soil	347a	400a	167b
mg N g^{-1} total C	3.93a	4.84b	3.20c
mg N g^{-1} total N	39.6a	57.3b	35.8a

In the Cropland soil, the moisture content at the time of sampling was already 60% of field capacity, and therefore only three moisture contents were considered (60, 80 and 100% of field capacity, corresponding to 25, 34 and 42 g H_2O 100 g^{-1} soil, respectively). Once wetted, the soil samples were placed in plastic containers at temperatures of 10, 15, 20, 25, 30 and 35 °C, and maintained thus for 42 days. Three different containers were prepared for each soil, moisture content and temperature. The containers were weighed twice every week to determine any weight loss (assumed to be due to loss of water), and distilled water was sprayed evenly over the surface of the soils to compensate for any loss of water. On day 0 (start of the experiment) and 3, 11, 24 and 42 days later, samples of soil were removed from each container to measure the concentrations of inorganic nitrogen.

The inorganic nitrogen was extracted with 2M KCl. Total inorganic nitrogen was determined by Kjeldahl distillation [18]. The data are expressed as net mineralized nitrogen (*Nmin*), calculated as the difference between the total inorganic nitrogen determined after the incubation time and the inorganic nitrogen at time 0 (i.e. at the start of the incubation period).

Soil pH was determined in water (ratio 1:2.5), particle size distribution by Robinson's pipette method, total carbon by a modification of Sauerlandt's method, and total nitrogen by Kjeldahl's method [17].

2.3. Kinetic models for the study of net nitrogen mineralization

The single compartment model of Stanford and Smith [8], one of the models most commonly applied in kinetic analysis of net nitrogen mineralization [11], was used to investigate nitrogen mineralization in the soils throughout the incubation period. The model is usually expressed in the form:

$$Nmin = N_o\left(1 - \exp^{-kt}\right) \quad (1)$$

where N_o represents the potentially mineralizable nitrogen (mg of N kg^{-1} soil) and k the mineralization constant (or mineralization rate), which is expressed in $days^{-1}$ because the incubation time (t) is expressed in days.

Of the different options available for resolving the above equation [11], we chose the option in which N_o is constant (for each soil and any combination of moisture and temperature) and k varies depending on the incubation conditions. This eliminated the confounding relationship between N_o and k and precluded the effect of incubation time on estimation of N_o [11]. To obtain N_o, the method described by [19] was followed: for the most extreme incubation conditions (temperatures of 30 and 35 °C and moisture contents of 80 or 100% of field capacity), non linear correlation was used to estimate the value of N_o, and successive iterations were applied to refine the estimate. This refined value, considered as the real value of N_o, was used in equation (1) to calculate the mineralization rate (k) under the different incubation conditions [11].

2.4. Influence of temperature on net nitrogen mineralization

The mineralization constant (k) values obtained for each soil and moisture content were fitted to the Arrhenius equation to determine the effect of temperature (T) on net nitrogen mineralization:

$$k = a \exp^{(bt)} \quad (2)$$

Logarithmic transformation of the previous equation ($\ln k = \ln a + b\mathrm{T}$) enabled parameters a and b to be obtained. Once the values of b were known, Q_{10} was calculated from the following expression:

$$Q_{10} = e^{10b} \quad (3)$$

in which it is assumed that the value of Q_{10} is constant throughout the entire range of temperatures considered.

Some authors [2] proposed the use of the Arrhenius model with a reference temperature that would serve to compare the variations in the rate of mineralization at this temperature with those at other temperatures. This enables calculation of the Q_{10} values for different temperatures, by use of the following expression:

$$Q_{10}^{[(\mathrm{T}-\mathrm{T}_1)/10]} = k/k_1 \quad (4)$$

where T is a temperature value, T_1, the reference temperature (in this case 20 °C), k the rate of mineralization at temperature T, and k_1 the rate of mineralization at the reference temperature.

2.5. Mathematical models for studying the effect of moisture on net nitrogen mineralization

To study the effect of moisture (M, % field capacity) on net nitrogen mineralization, the mineralization constant (k) values obtained at each temperature were fitted to the following equations, selected for their simplicity and because they are widely used [2,9]:

$$\text{Linear} \quad k = a + q_m\,\mathrm{M} \quad (5)$$

$$\text{Semilogarithmic} \quad k = a + q_m \log \mathrm{M} \quad (6)$$

$$\text{Partial parabolic} \quad k = a + q_m\,\mathrm{M}^2 \quad (7)$$

$$\text{Complete parabolic} \quad k = a + q_m\,\mathrm{M} + q'_m\,\mathrm{M}^2 \quad (8)$$

where q_m and q'_m are the coefficients of dependency of net nitrogen mineralization on moisture.

2.6. Interaction between temperature, moisture and incubation time

To predict the effect of combined variations in moisture and temperature on net nitrogen mineralization, the net mineralization data were fitted to different types of equations in which the variables temperature, moisture content and incubation time (and the combinations of these variables) appear in a linear, quadratic or logarithmic form (equations not shown). One of these equations was selected for its simplicity and because it provided good fits for all three soils used in the study. Only the linear forms of the variables time of incubation (***t***), moisture (**M**) and temperature (**T**) were included in the equation selected, along with another variable that represents the combined interaction between the above three parameters:

$$Nmin = z + a_1 t + a_2\mathrm{M} + a_3\mathrm{T} + a_4\mathrm{T}t\mathrm{M} \quad (9)$$

2.7. Statistical analyses

Nitrogen determinations were carried out in triplicate throughout the incubation, and therefore the individual values for each soil, moisture level, temperature and time of incubation are mean values from 9 determinations (3 replicates in the incubation × 3 replicates in the determination). Mean values and deviations, tests of significance of means (Tukey's test), the iteration procedure for calculation of N_0, and fits to the different equations cited in the study were carried out with the Statistica 6.0 programme (StatSoft©) for Windows (StatSoft Inc., 2001).

3. Results and discussion

3.1. Net nitrogen mineralization during the incubation period

In the Forest soil, net mineralization was observed for all conditions of moisture and temperature, whereas in the Grassland and Cropland soils net immobilization was observed during the early stages of the incubation at some temperatures and moisture contents (Figs. 1 and 2). The initial immobilization was low, except in the Grassland soil at 35 °C. The high degree of immobilization in the latter soil can be explained by considering that at this high temperature the microbial metabolism is initially so intense that the microbial consumption of inorganic nitrogen is higher than that generated by mineralization of organic nitrogen [6]. However, the fact that the immobilization was only produced at some of the moisture contents studied, raises some doubts about this

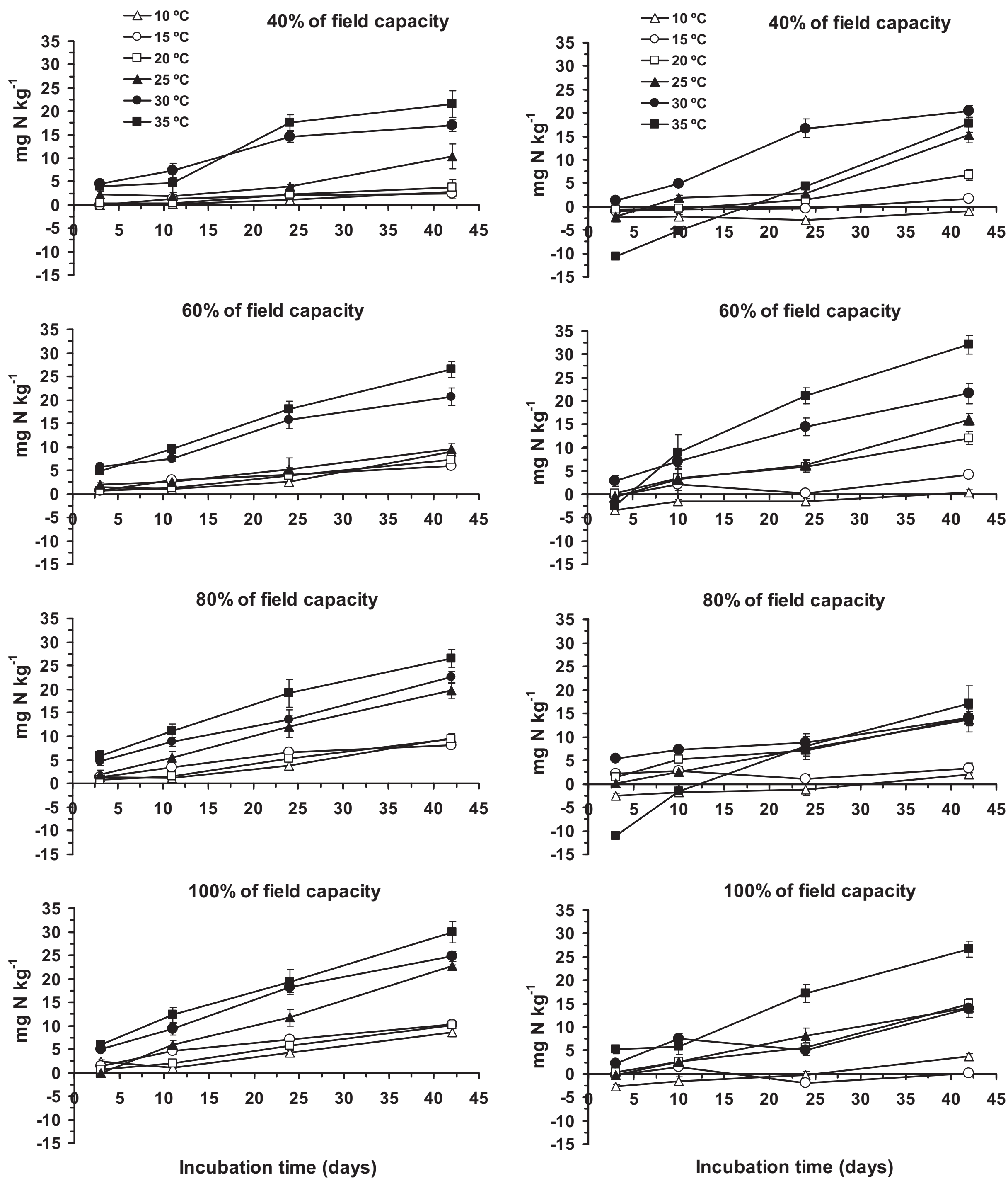

Fig. 1. Variation in the values of mineralized nitrogen throughout the incubation for the different conditions of moisture and temperature in Forest and Grassland soils. For each incubation time, mineralized nitrogen (*Nmin*) was calculated as the difference between the total inorganic nitrogen determined after the incubation time and the inorganic nitrogen present at time 0 (i.e. at the start of the incubation period).

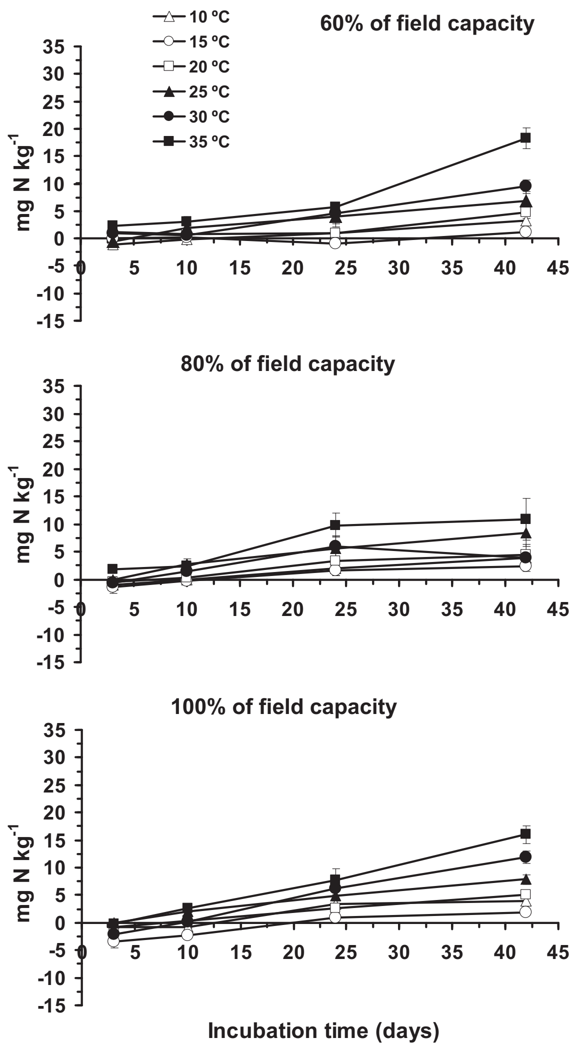

Fig. 2. Variation in the values of mineralized nitrogen throughout the incubation for the different conditions of moisture and temperature in Cropland soil. For each incubation time, mineralized nitrogen (*Nmin*) was calculated as the difference between the total inorganic nitrogen determined after the incubation time and the inorganic nitroeng present at time 0 (i.e. at the start of the incubation period).

interpretation. In any case, in all soils the amount of net mineralized nitrogen increased with the duration of incubation, and generally also increased with the temperature of incubation. The effect of moisture was not as clear, although in the Forest soil the amount of net mineralized nitrogen increased with moisture content, particularly at low incubation temperatures. The response of the Grassland and Cropland soils to soil moisture was more complex, but mineralization did not increase with increased moisture content. The fact that the effect of moisture on nitrogen mineralization was lower than that of the temperature has been reported by other authors [12,13], who have suggested that edaphic moisture is less limiting than temperature for the microorganisms responsible for mineralization.

3.2. *Potentially mineralizable nitrogen (N_o)*

The values of potentially mineralizable nitrogen, N_o (expressed as absolute values, per unit of total carbon and per unit of total nitrogen), are shown in Table 1. The highest values of N_o were always observed in the Grassland soil and the lowest values in the Cropland soil. The higher values of N_o in the Grassland soil suggest that the successive inputs via the slurry added to the Grassland soil may have favoured an increase in N_o, because the slurries used as fertilizers contain large amounts of readily mineralizable nitrogen [20].

3.3. *Influence of temperature and moisture on net nitrogen mineralization*

For the Forest soil, first order mono-compartmental kinetics (Equation (1)) were verified under all incubation conditions, although the best fits were obtained for temperatures above 15 °C (Table 2). A different situation was observed in the Grassland soil (Table 2) as the experimental data generally only fitted the mono-compartment model at the intermediate range of temperature. In the Cropland soil, the mono-compartment model provided a rather good fit for the experimental data for all incubation conditions, except at 15 °C (Table 2).

The mineralization constant (k) values obtained in the present study (Table 2) are within the range of values reported in both classical [8] and more recent studies [11,19]. In general, the values of k increased with increasing temperature and moisture in the Forest soil, whereas they only increased with increasing temperature in the Grassland and Cropland soils, and the moisture content did not appear to have an important effect on the mineralization process (Table 2). The rate of mineralization increased sharply with temperature, particularly between 25 and 35 °C. Such increases have been interpreted as being caused by the rapid consumption of readily mineralizable organic matter [19], although the acceleration of mineralization with temperature is now interpreted as the result

Table 2
Values of k (days^{-1}) obtained from the fit of the net mineralization data (*Nmin*) throughout the incubation (t, days) to the equation (1), $Nmin = N_o\,(1-e^{(-k)t})$. N_o, potentially mineralizable nitrogen (see Table 1). Values of Q_{10} and R^2 obtained from equation (3): for each soil, Q_{10} values preceded by the same capital letter are not significantly different ($P < 0.01$); for each moisture content, Q_{10} values followed by the same lower case letter are not significantly different between the different soils ($P < 0.05$).

Soil	%Moisture	10 °C		15 °C		20 °C		25 °C		30 °C		35 °C		Q_{10}	R^2
		R^2	k	R^2	k	R^2	k	R^2	k	R^2	k	R^2	k		
Forest	40%	0.91**	0.0018	0.76	0.0020	0.95***	0.0026	0.88*	0.0075	0.86*	0.0193	0.94***	0.0245	A3.32a	0.93**
	60%	0.82	0.0058	0.86*	0.0049	0.96***	0.0053	0.95***	0.0074	0.92**	0.0232	0.97***	0.0326	B2.88a	0.90**
	80%	0.90**	0.0067	0.87*	0.0072	0.98****	0.0072	0.99****	0.0190	0.94***	0.0243	0.97***	0.0357	C2.02a	0.98****
	100%	0.86*	0.0063	0.91**	0.0091	0.99****	0.0078	0.96***	0.0212	0.98****	0.0310	0.96***	0.0408	C2.16a	0.99****
Grassland	40%	n.f.	n.f.	0.74	0.0033	0.74	0.0082	0.96***	0.0183	n.f.	n.f.	n.f.	n.f.	A5.16b	0.99****
	60%	n.f.	n.f.	n.f.	n.f.	0.97***	0.0078	0.91**	0.0103	0.99****	0.0187	0.93**	0.0314	B2.77a	0.99****
	80%	n.f.	n.f.	n.f.	n.f.	0.95***	0.0099	0.97***	0.0092	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.
	100%	n.f.	n.f.	n.f.	n.f.	0.91**	0.0093	0.97***	0.0098	n.f.	n.f.	0.95***	0.0240	C2.09a	0.97***
Cropland	60%	0.70	0.0040	n.f.	n.f.	0.70	0.0062	0.95***	0.0120	0.88*	0.0160	0.72	0.0355	A3.27b	0.97***
	80%	0.81	0.0054	n.f.	n.f.	0.90**	0.0076	0.98****	0.0169	n.f.	n.f.	0.90**	0.0278	B1.97a	0.96***
	100%	0.72	0.0064	n.f.	n.f.	0.88*	0.0075	0.98****	0.0147	0.80	0.0207	0.86*	0.0333	B2.33a	0.98****

****, $P < 0.001$; ***, $P < 0.05$; **, $P < 0.01$; *, $P < 0.5$; *n.f.*, no fit.

Table 3
Values of Q_{10} calculated for the different temperatures and moisture conditions during the incubation, with 20 °C considered as the reference temperature (Equation (4)). *n.f.* not fitted. For each soil, Q_{10} values preceded by the same capital letter are not significantly different ($P < 0.01$); for each moisture content, Q_{10} values followed by the same lower case letter are not significantly different between the different soils ($P < 0.05$).

Soil	% M	10 °C	15 °C	25 °C	30 °C	35 °C
Forest	40	A1.47a	A1.77	A8.14a	A7.37a	A4.44
	60	n.f.	B1.17	B1.95a	B4.78a	B3.35a
	80	B1.08a	n.f.	C6.96a	C3.37	C2.88a
	100	B1.25a	n.f.	C7.32a	C3.41a	C2.72a
Grassland	40	n.f.	n.f.	A6.08b	A5.45b	n.f.
	60	n.f.	n.f.	B1.65a	B2.34b	A2.49b
	80	n.f.	n.f.	n.f.	n.f.	n.f.
	100	n.f.	n.f.	C1.14b	n.f.	B1.88b
Cropland	60	A1.54	n.f.	A3.74b	A2.56b	A3.19c
	80	A1.40b	n.f.	B4.95b	n.f.	B2.37b
	100	B1.17a	n.f.	A3.84c	A2.76b	C2.70a

of greater depolymerization of organic matter due to more intense activity of the exocellular enzymes that generate free monomeric units, such as sugars and amino acids [6]. This hypothesis, which forms part of the so-called *new paradigm of nitrogen mineralization* [6], appears to be confirmed in this case, as it has been observed that the main enzymes that catalyze the first stages of organic matter decomposition (cellulases, proteases) display a strong increase in activity between 30 and 50 °C [16]. In addition, the fact that the microorganisms that decompose organic matter act at a faster rate at 35 °C than at lower temperatures [21] also favours increased mineralization with temperature.

The temperature-related variations in the k values in all three soils follow the Arrhenius exponential model, which enables estimation of the value of Q_{10} for almost all incubation conditions (Equation (3)). With the exception of the Q_{10} value in the Grassland soil at the lowest moisture content (40%), as commented further below, the Q_{10} values ranged between 3.32 and 1.97 (Table 2), i.e. close to 2, which is the value considered as physiologically normal [2,10]. The Q_{10} value obtained for the lowest moisture content in the Grassland soil (5.16, Table 2) was higher than for the other two soils at any moisture level, although this may be an anomaly caused by the small number of points used in the fit (only 3), as it was not possible to include the rate of mineralization at various temperatures (10, 15 and 35 °C) in the calculation. We therefore consider that this Q_{10} value is of little use for describing the effect of temperature on net nitrogen mineralization at this moisture content.

The strong increase in the rate of mineralization at the highest temperatures tested suggests that the relation between mineralization and temperature may differ from that predicted under the assumption of a constant Q_{10} rule. Several researchers, such as Rodrigo et al. [2] and Harthey and Ineson [22], have proposed the use of the Arrhenius model at a reference temperature (usually 20 °C), for comparison of the variations in the rate of mineralization at this temperature with those at other temperatures (Equation (4)). Thus, in the Forest and Cropland soils (for which it was possible to calculate several Q_{10} values), the highest sensitivity values tended to be obtained at 25 °C (Table 3). At lower temperatures, the sensitivity decreased sharply and although there was also a decrease at higher temperatures, this was less evident. Furthermore, of these two soils, the Cropland soil tended to be the least sensitive to temperature. The small number of Q_{10} values that could be calculated for the Grassland soil showed that, unlike in other soils, in this soil the sensitivity to mineralization generally increased with temperature. The different behaviour of the Grassland soil suggests that the microbial community will be enriched with thermophilic microorganisms as a result of the addition of slurry, in which this type of microbiota predominates [23].

Nitrogen mineralization was also sensitive to changes in soil moisture (Table 4). For the Forest soil at all temperatures studied, the rates of net nitrogen mineralization fitted well to all four equations considered, with high regression coefficients, although the best fits were obtained with the complete parabolic equation (Equation (8)). In contrast, very few significant fits were obtained for the mineralization constants and moisture in the Grassland and Cropland soils (Table 4); some of the equations explained the variation in the mineralization constants with soil moisture, but only at some temperatures. In any case, in all three soils increases in the mineralization constants and decreases in Q_{10} values were observed for moisture contents up to 80%. In addition, the values of

Table 4
Fits of the mineralization constants to different equations in order to study the dependence on moisture.

Soil	Linear		Semilogarithimic		Partial parabolic		Complete parabolic			
	R^2	q_m	R^2	q_m	R^2	q_m	R^2	a	q_m	q'_m
Forest										
10 °C	0.86*	0.000074	0.93**	0.011733	0.78	0.000000	1.00****	−0.009631	0.000383	−0.000002
15 °C	0.99****	0.000119	0.99****	0.017340	0.98****	0.000001	0.99****	−0.002975	0.000129	−0.000001
20 °C	0.98****	0.000089	0.99****	0.013261	0.94***	0.000001	0.99****	−0.004286	0.000209	−0.000010
25 °C	0.89*	0.000253	0.84*	0.035513	0.90**	0.000002	0.90**	0.007191	−0.000115	0.000003
30 °C	0.96***	0.000182	0.92**	0.025708	0.98****	0.000001	0.99****	0.020670	−0.000114	0.000002
35 °C	0.99****	0.000265	0.99****	0.038941	0.98****	0.000002	1.00****	0.0190906	0.000384	−0.000001
Grassland										
10 °C	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.
15 °C	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.
20 °C	0.89*	0.000105	0.94***	0.016577	0.82	0.000001	0.99****	−0.011692	0.000481	−0.000100
25 °C	0.61	0.000022	0.68	0.003601	0.54	0.000000	0.78	0.003882	0.000148	−0.000100
30 °C	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.
35 °C	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.
Cropland										
10 °C	0.99****	0.000060	1.00****	0.010836	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.
15 °C	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.
20 °C	0.83	0.000033	0.87*	0.006112	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.
25 °C	0.55	0.000068	0.61	0.013447	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.
30 °C	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.d.
35 °C	0.28	−0.000055	0.35	−0.012357	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.

****, $P < 0.001$; ***, $P < 0.005$; **, $P < 0.01$; *, $P < 0.05$; *n.f.*, not fit.

both parameters for moisture contents of 80 and 100% were similar in all three soils, and not significantly different from each other ($P < 0.001$, Table 2). The similar response of the rates of mineralization at the highest moisture contents makes it difficult to develop simple models, as obviously fewer points can be used in the fits that describe the effects of soil moisture on net nitrogen mineralization. This was clear for the Grassland and Cropland soils, for which the model fits were always very poor, in contrast to the fits for the Forest soil, which were relatively good (Table 4). The agricultural soils may behave differently from the Forest soil because the microorganisms in agricultural soils are capable of utilizing the water content in semi-decomposed organic waste supplied by organic fertilizers [24]. Evidently, the lack of significant fits for all of the soils prevents clear conclusions being reached with regard to the dependence of net nitrogen mineralization on soil moisture, as also reported by other authors [11,25].

3.4. Influence on soil use on net nitrogen mineralization

One of the aims of the study was to investigate the influence of soil use on net nitrogen mineralization. However, the data obtained were not clear in this respect. Thus, although the net mineralization values were different in each of the soils (Figs. 1 and 2), in most cases the constant mineralization values (for the same moisture and temperature conditions) for the three soils were of the same magnitude (Table 2). This suggests that, independently of the organic matter content and the size of mineralizable nitrogen pool, mineralization occurs in a similar way in all soils and at remarkably similar rates. However, the influence of temperature and moisture on the mineralization constant varied depending on soil use. Thus, the Q_{10} values obtained from equation (4) (Table 3) revealed that the Forest and Cropland soils behave similarly, with decreases in the value of Q_{10} at temperatures above 25 °C. The behaviour of the Grassland soil was different as the Q_{10} value increased with the incubation temperature, possibly due to the different type of mineralizable organic matter in this soil, as a result of the application of organic fertilizers such as cattle slurry. Nonetheless, confirmation that grassland use modifies the temperature sensitivity of soil organic matter requires analysis of more soils, as well as the use of more complex models than the Stanford and Smith model.

There were also differences between soils as regards moisture-related effects. The Forest soil, in which maximum mineralization occurred at 80% moisture content, provided good fits to all of the models used, whereas the Grassland and Cropland soils (in which the behaviour of mineralization in response to moisture was less clear), showed very poor fits (Table 4). The different behaviour of the soils suggests that the agricultural use modifies the behaviour in response to moisture of the pool of mineralizable nitrogen.

In summary, although some of the parameters deduced from the mineralization data, such as the kinetic constants, do not clearly indicate the influence of soil use, the sensitivity of these constants to temperature and moisture was higher in the Forest soil than in the agricultural soils. This suggests that climate change may have greater effects on nitrogen mineralization in forest soils than in cropped soils. However, the small number of soils studied prevents clear conclusions being reached with regard to the influence of soil use on the modification of soil nitrogen mineralization by changes in temperature or moisture.

3.5. Interaction between temperature and moisture on soil net nitrogen mineralization

As initially indicated, climate change involved simultaneous changes in the temperature and moisture regimes of soils, although these modifications will differ for each of the parameters, depending on the location of the soil and the time of year. Therefore, in addition to determining the influence of temperature and of moisture on net nitrogen mineralization, the influence of the interaction between these two factors must also be determined. Although there is no general agreement as to whether or not this interaction actually exists [25,26], Sierra [13] attempted to demonstrate its existence by plotting the rates of mineralization at different temperatures and moisture contents and considering that if non parallel lines were obtained, this would indicate a certain degree of dependence between moisture and temperature. The estimate indicated by Sierra [13] was used here, considering the Q_{10} values obtained in equation (9) (Table 3) for the different moisture contents and temperatures of 10, 30 and 35 °C; for the Forest soil the slopes of the lines differed at different temperatures, with shallow slopes at low temperatures (10 °C) and much steeper slopes at high temperatures (Fig. 3). Such lines were not obtained for the Grassland soil, but were obtained for the Cropland soil at extreme temperatures (10 and 35 °C). However, for this soil and unlike those obtained for the Forest soil, the lines were almost parallel. In other words, it is not clear whether there is an interaction between temperature and moisture. This uncertainty may also be due to the fact that Q_{10} cannot provide a complete explanation of the response of soil nitrogen mineralization when the soil moisture status is not known [13]. In any case, the above results indicate that if such an interaction exists, it would depend on the type of soil organic matter, i.e. on soil use and management.

Given the above-mentioned problems, the study of the combined effects of temperature and moisture requires the use of equations with no physical basis. In the present study, the equation considered (Equation (9)) provided significant fits (high values of R^2, low residual values) to the experimental data. The regression coefficient (R^2) was higher for the Forest soil than for the other two soils (Table 4), which again suggests that the agricultural use of the

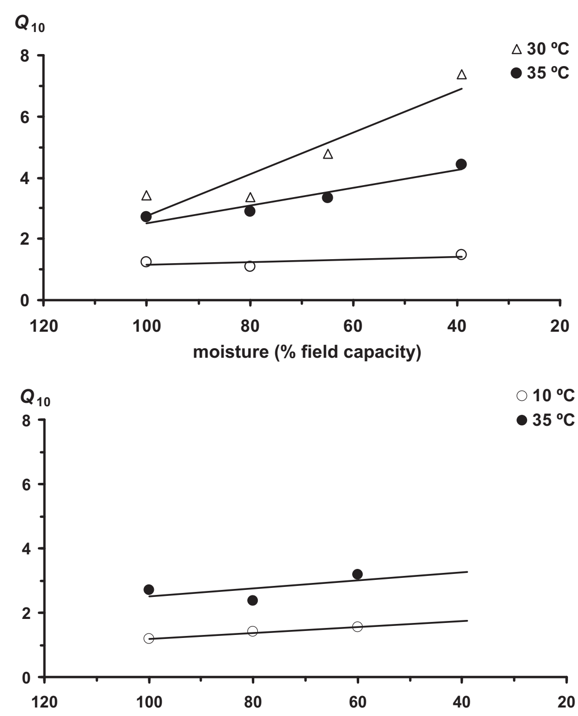

Fig. 3. Q_{10} values obtained in equation (4) for Forest and Cropland soil for different moisture and temperature conditions.

Table 5
Values of the regression coefficients and for the multiple equation (Equation (9)), $Nmin = z + a_1 t + a_2 \mathrm{M} + a_3 \mathrm{T} + a_4 \mathrm{T}t\mathrm{M}$. Parameters of the equation followed by the same lower case letter are not significantly different between the different soils ($P < 0.05$).

Soil	R^2	z	a_1 ($\times 10^{-4}$)	a_2 ($\times 10^{-4}$)	a_3 ($\times 10^{-3}$)	a_4 ($\times 10^{-6}$)
Forest	0.88***	−0.05a	−4.90a	−7.49a	5.68a	5.76a
Grassland	0.71**	−0.03a	−17.50b	−10.25b	5.12a	2.50b
Cropland	0.83***	0.04a	−28.74c	−11.38b	2.22b	2.39b

***, $P < 0.005$; **, $P < 0.01$.

soils modifies net nitrogen mineralization. The independent term was close to zero in these three soils, and in all soils the coefficients of the same variable were affected by the same sign and were of the same magnitude (Table 5), suggesting that some factor (possibly related to geographical or mesoclimatic-type aspects) regulates net nitrogen mineralization in the soils in the area. The most significant term from a statistical point of view is *a4* (coefficient of the TtM interaction), which was positive for all three soils, suggesting the existence of a positive interaction between temperature and moisture, which increases over time, in accordance with the heat-sum concept described by Honeycutt et al. [27].

The fact that good fits to equation (9) were obtained for all three soils indicates that this may be a simple way of estimating how climate change affects soil nitrogen dynamics, as the equation would enable quantification of the influence of changes in temperature and moisture during different periods. However, it must be taken into account that the use of this equation is seriously limited as the soil nitrogen cycle is extraordinarily complex (at least the gross mineralization and immobilization should be differentiated, and possible losses via emissions should be estimated), and this complexity is not represented in the equation. Furthermore, the fact that the incubations must be of short duration to minimize any distortions in the characteristics of the soil organic matter implies that the proportion of total nitrogen affected by the incubation will be very small, and no information will be obtained about the transformations that climate change may have on a large part of the soil nitrogen.

4. Conclusions

Temperature and moisture affected net nitrogen mineralization in the three soils studied, although the response of the Forest soil was more straightforward than that of the agricultural soils.

For all three soils the sensitivity of net nitrogen mineralization to temperature was maximal at 25 °C. The optimal moisture content for nitrogen mineralization was 80% of the field capacity and mineralization at 100% of field capacity was only slightly lower than that obtained at 80%. In general, moisture had less effect than temperature on net nitrogen mineralization.

Modelling of nitrogen mineralization in relation to soil temperature and moisture is difficult in the soils under study, although it appears to be highly dependent on a triple interaction between temperature, moisture and time.

Further studies must be carried out in order to determine the extent to which changes in the quality of organic matter, caused by land use, affect any modifications to soil nitrogen that may be generated by climate change.

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