

## TEMPERATURE AND SOIL MOISTURE DEPENDENCE OF N MINERALIZATION IN INTACT SOIL CORES

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**Summary**—The combined effect of temperature (10, 20 and 25°C) and soil moisture (30, 200 and 1700 kPa) on net N mineralization was examined using undisturbed soil samples in laboratory incubations. Simulations with a mechanistic model of soil anaerobiosis were made to assess the effect of temperature and soil moisture on soil aeration, which also affects N mineralization. The rate of net N mineralization showed a high variability, and 57% of this variation was associated with factors other than temperature and moisture. N mineralization was more responsive to temperature than it was to moisture. Two models based on the Arrhenius and the  $Q_{10}$  functions described well the experimental data and indicated a temperature–moisture interaction affecting N mineralization. When the measured rates were corrected to take into account only the calculated aerobic fraction of the soil, a good fit with both models was still obtained. This implies that the Arrhenius and the  $Q_{10}$  functions do not discriminate between the direct effect of temperature on N mineralization and the indirect effect associated to soil aeration. The use of these functions to describe N mineralization in undisturbed samples requires information on the aeration status of the soil. © 1997 Elsevier Science Ltd. All rights reserved

### INTRODUCTION

Temperature and moisture are the major environmental factors affecting N mineralization in soils. Most of the studies dealing with the effect of these factors on N mineralization have been carried out by incubating sieved soil samples (dried or field-moist soils) under different temperature or soil moisture conditions (e.g. Stanford and Epstein, 1974; Addiscott, 1983; Ellert and Bettany, 1992). Aeration in such disturbed soil may be more favourable for N mineralization than in undisturbed soil and, consequently, the response may be biased.

In field conditions, soil moisture affects N mineralization in a direct and indirect way. The direct effect is related to the water availability for microbial activity, and in this case it is suitable to express soil moisture in terms of water potential (Orchard and Cook, 1983). Water also affects N mineralization by controlling  $O_2$  diffusion within the soil and the volume of soil supporting aerobic microbial activity. To analyse this, soil water content is currently expressed in terms of water-filled pore space (WFP) (Skopp *et al.*, 1990). Similarly, temperature directly controls N mineralization by affecting the biochemical processes and, indirectly, by affecting  $O_2$  consumption by microorganisms and the aerobic volume of the soil (Renault and

Sierra, 1994). As a consequence, when incubations are performed using sieved soil, the indirect effect of temperature and moisture are not taken into account because small aggregates (usually <2 mm) are less favourable to display anaerobic conditions (Sierra *et al.*, 1995). A better approach to assess the real effect of temperature and soil moisture on N mineralization may be obtained by using intact soil cores (Raison *et al.*, 1987). An important aspect concerning the use of undisturbed samples is the large uncertainty introduced by the spatial variability of the biological and the physical factors involved in N mineralization (Sierra, 1996). For example, differences of soil structure between samples (e.g. bulk density) may induce large differences in the aerobic volume of the soil, even at the same gravimetric water content.

The aim of this study was to test the ability of two simple models (based on the Arrhenius and the  $Q_{10}$  functions) to describe the response of N mineralization to temperature and soil moisture in undisturbed soil samples. The indirect effects of temperature and moisture on soil N mineralization were also analysed to know how they may modify the usefulness of the tested models.

### MATERIALS AND METHODS

#### *Soil properties and laboratory incubations*

The soil used in this study was a Typical Argudoll from the Pergamino Experimental Station

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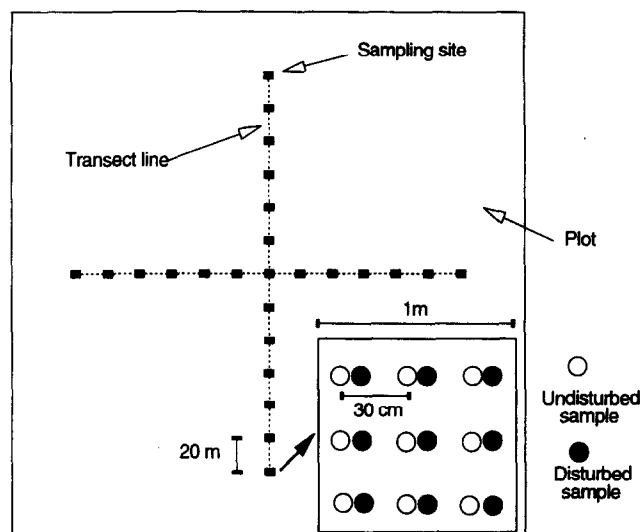


Fig. 1. Sampling design.

(Argentina) (33°50'S, 60°31'W). The soil had been maintained under a wheat-fallow rotation during the previous 5 y. The characteristics of the Ap horizon were: texture clay loam, bulk density  $1.2 \text{ Mg m}^{-3}$ , pH ( $\text{H}_2\text{O}$ ) 6.1, organic C  $21.8 \text{ g kg}^{-1}$ , organic N  $2.2 \text{ g kg}^{-1}$ . Twenty-five sampling sites were located on two perpendicular transects (Fig. 1) and nine pairs of samples were extracted at each site: one sample of the pair was used to provide the initial data of mineral N content and gravimetric water content (disturbed sample), the adjacent sample was taken for incubation in laboratory (undisturbed sample). The undisturbed samples were extracted using PVC cylinders (5 cm deep, 6.5 cm external dia, 0.2 cm of wall thickness). The disturbed samples were taken with a sampler (5 cm deep, 4 cm dia). All of the soil samples were stored at approximately  $3^\circ\text{C}$  until use (about 72 h).

The laboratory experiment consisted of 25 replicates of a  $3^2$  factorial of temperature (10, 20 and  $25^\circ\text{C}$ ) and soil moisture (30, 200 and 1700 kPa). The lowest and the highest temperature were selected to represent the mean daily temperature at 10 cm depth of the coldest and the hottest months, respectively. The values of water potential corresponded to gravimetric water contents of 0.280, 0.221 and  $0.166 \text{ kg kg}^{-1}$ , respectively. The nine temperature-moisture treatments were randomly distributed to the undisturbed samples of each sampling site. The water content of each soil sample was adjusted gravimetrically based on the water content of the adjacent disturbed sample. As the initial water content was extremely low (mean  $0.13 \text{ kg kg}^{-1}$ ), distilled water was added to obtain the required moisture content. Water was gently dripped on the top surface of the soil core with a syringe. The soil was incubated for 35 d. Water loss during incubations was negligible. Nitrate-N were extracted in 2M KCl and determined colorimetri-

cally by hydrazine reduction (Kampshake *et al.*, 1967) in the disturbed samples as well as in the intact soil cores at the end of the experiment. Net N mineralization was estimated as the difference between the initial and the final  $\text{NO}_3\text{-N}$  content. The  $\text{NH}_4\text{-N}$  content was not measured because preliminary work indicated that it represented less than 1% of the total mineral N content.

#### Temperature and moisture response functions

Both the Arrhenius and the  $Q_{10}$  functions were tested to describe the effect of temperature on N mineralization. The Arrhenius equation is

$$k = k_1 e^{-B/T} \quad (1)$$

and the  $Q_{10}$  equation is

$$k = k_2 Q_{10}^{T/10} \quad (2)$$

where  $k$  ( $\text{d}^{-1}$ ) is the constant rate of N mineralization,  $T$  ( $^\circ\text{K}$ ) is the absolute temperature,  $k_1$  and  $k_2$  are coefficients,  $B$  ( $^\circ\text{K}$ ) is the ratio of the activation energy  $E$  ( $\text{J mol}^{-1}$ ) to the ideal gas constant  $R$  ( $8.3143 \text{ J mol}^{-1} \text{ K}^{-1}$ ), and  $Q_{10}$  is the ratio of constant rates measured at temperatures differing by  $10^\circ\text{C}$ . In this study, we used equations (1) and (2) to describe the rate of N mineralization  $R_N$  ( $\text{mg kg}^{-1} \text{ d}^{-1}$ ) and not the constant rate  $k$ . By considering a first-order kinetic model (Stanford *et al.*, 1973), the relation between  $R_N$  and  $k$  is

$$R_N = k \times N \quad (3)$$

where  $N$  ( $\text{mg kg}^{-1}$ ) is the amount of mineralizable N. Then, to use equations (1) and (2), the coefficients  $k_1$  and  $k_2$  have to be replaced by

$$k_i' = k_i \times N. \quad (4)$$

Therefore, neither  $B$  nor  $Q_{10}$  are modified in relation to the original form of the equations.

To quantify the effect of soil moisture on N mineralization, we considered  $k_1'$  and  $k_2'$  as depending on soil moisture and fitted a function which agreed well with the experimental data. The functions were

$$\log k_1' = \log m + n \log \Psi \quad (5)$$

and

$$\log k_2' = \log p + q \log \Psi \quad (6)$$

where  $m$ ,  $n$ ,  $p$  and  $q$  are empirical constants, and  $\Psi$  (kPa) is the water potential. Similar linear relationships as those of equations (5) and (6) were reported by Stanford and Epstein (1974) for N mineralization, and by Orchard and Cook (1983) for soil respiration. The combined effects of temperature and moisture were then described as

$$R_N = m \Psi^n e^{(-B/T)} \quad (7)$$

for the Arrhenius function, and

$$R_N = p \Psi^q Q_{10}^{(T/10)} \quad (8)$$

for the  $Q_{10}$  function. Experimental data was fitted to equations (7) and (8) using the non-linear iterative procedure proposed by Bard (1974).

#### *Estimate of the anaerobic fraction of the soil*

To assess how the indirect effects of temperature and soil moisture modify the relationships between experimental and modelled data, we carried out some simulations to estimate the anaerobic fraction of the soil by using the model of  $O_2$  diffusion proposed by Renault and Sierra (1994). This model calculates anaerobiosis taking into account the physical (e.g.  $O_2$  diffusion between and within aggregates) as well as the biological variables (e.g. soil respiration) affecting  $O_2$  distribution in the soil. The value of most of the parameters of the model are unknown for the soil used in this study (e.g. distribution of aggregate size, coefficient of  $O_2$  diffusion). Thus, the aim of this analysis was not to estimate the actual anaerobic fraction of the soil, but to examine qualitatively the effect of the presence of anaerobiosis on the observed response of N mineralization to temperature and soil moisture for the range tested in this study.

The parameters were set as follows: the rate of  $O_2$  consumption was  $2.5 \times 10^{-5} \text{ mol m}^{-3} \text{ s}^{-1}$  at  $20^\circ\text{C}$ , the coefficient of  $O_2$  diffusion was  $5 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$  for saturated aggregates. The soil bulk density was  $1.2 \text{ Mg m}^{-3}$ , the aggregate density was assumed to be  $1.6 \text{ Mg m}^{-3}$  and the particle density  $2.75 \text{ Mg m}^{-3}$ . All of these parameters values are typically observed in soil (Renault and Sierra, 1994; Sierra *et al.*, 1995). The calculations were made by considering an external  $O_2$  concentration of  $0.21 \text{ m}^3 \text{ m}^{-3}$  and a soil depth of 5 cm (depth of a soil sample). With the assumed values of soil density it may be calculated that the aggregates were

fully saturated at a gravimetric water content of  $0.262 \text{ kg kg}^{-1}$  (WFP 55.7%). It was then assumed that only for the highest water content tested in this study ( $0.280 \text{ kg kg}^{-1}$ , WFP 59.6%) the aggregates were saturated and anaerobiosis might occur. For unsaturated aggregates, it may be assumed that  $O_2$  diffusion was not limited by water and they were fully aerobic.

## RESULTS AND DISCUSSION

### *Response of N mineralization*

For the range of the factors tested in this study, N mineralization increased continuously with the increase of temperature and the decrease of water potential (Fig. 2). Some authors suggested that the effect of temperature on N mineralization depend on the temperature regime of the soil because of the physiological adaptation of the organisms to their soil habitat (Ellert and Bettany, 1992; Grundmann *et al.*, 1995). Grundmann *et al.* (1995) observed that the optimal temperature for N mineralization of two soils ( $25$  and  $20^\circ\text{C}$ ) was close to the average soil temperature in summer ( $25$  and  $22^\circ\text{C}$ , respectively). Conversely, most of the authors found that N mineralization increased continuously in the range from  $5$  to  $35^\circ\text{C}$  without any optimal value of temperature (Stanford *et al.*, 1973; Addiscott, 1983; Ellert and Bettany, 1992). Concerning soil moisture, the smallest water potential tested in our study ( $30 \text{ kPa}$ ) was reported by several authors as the optimum water content for N mineralization (Stanford and Epstein, 1974; Macduff and White, 1985; Cabrera and Kissel, 1988). Consequently, the response of N mineralization in this study may be considered as placed in the deficiency-optimum range of soil moisture. Significant N mineralization occurred at a water potential as high as  $1700 \text{ kPa}$  (Fig. 2). For example, at  $25^\circ\text{C}$  the rate at  $1700 \text{ kPa}$  was about 65% of the rate at  $30 \text{ kPa}$ . This agrees with the results obtained by Stanford and Epstein (1974) and Bramley and White (1990) under laboratory conditions and Frazer *et al.* (1990) under field conditions. These results show that N mineralization does not cease at the plant wilting point ( $1500 \text{ kPa}$ ) as assumed in some models of N mineralization (Macduff and White, 1985; Grundmann *et al.*, 1995).

The rate of N mineralization showed high spatial variation (Fig. 2). The coefficient of variation decreased with the increase of the rate, i.e. 310% for the  $10^\circ\text{C}/1700 \text{ kPa}$  treatment (smallest rate), and 36% for the  $25^\circ\text{C}/30 \text{ kPa}$  treatment (highest rate). It seems that no controlled factors (e.g. amount of mineralizable substrate, Sierra, 1996; aeration status) might partially explain the observed spatial variation. However, the procedure to estimate the rate of N mineralization may also induce variability. For instance, differences in the initial mineral N

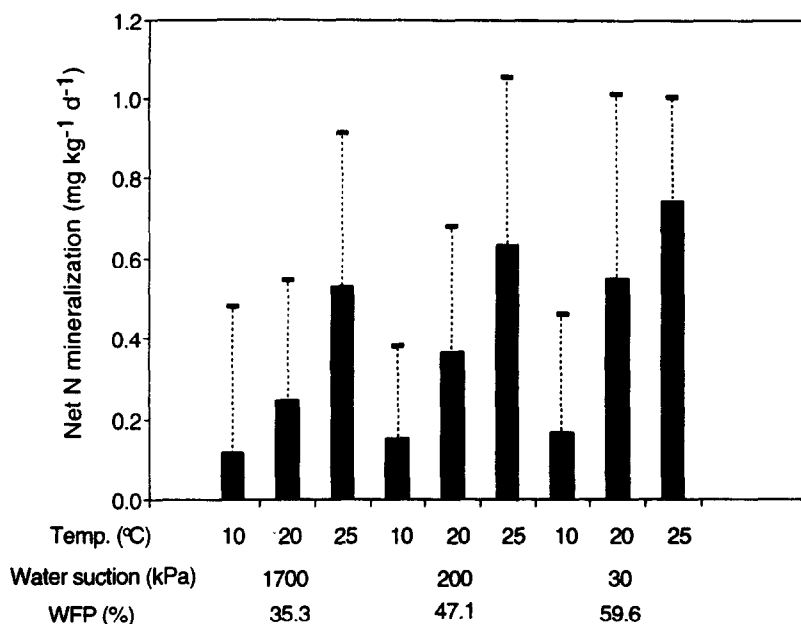


Fig. 2. Rates of net N mineralization for the nine temperature–soil moisture treatments. Vertical bars indicate standard error ( $n = 25$ ). WFP is the water filled porosity.

content between the disturbed sample and the adjacent intact soil core may increase the variance of the estimated rate (Sierra, 1996). This might also explain some negative values of N mineralization observed for the 10°C treatment; however, net N immobilization at low temperatures is another possible explanation (Frazer *et al.*, 1990).

To assess the effect of temperature and soil moisture we performed an ANOVA of the experiment as a factorial in randomized blocks; the blocks corresponding to the sampling sites (Fig. 1). The effect of temperature was higher than that of soil moisture, which may be also observed in Fig. 2 (calculated  $F$  for temperature effect = 41.55, calculated  $F$  for moisture effect = 5.27, tabulated  $F = 3.04$  for  $\alpha = 0.05$ ). The lower response to moisture might reflect the adaptation of microorganisms to the moisture regime of the soil. Because of the soil texture, which affects the hydraulic properties of the soil, high water potentials are reached in spite of a relatively high water content (i.e. 1700 kPa at 0.166 kg kg<sup>-1</sup>). As a consequence, abrupt changes of water potential may occur within a few days after a rain, principally in summer. Similar results were reported by Bramley and White (1990) for a soil supporting considerable variation of moisture status. It is interesting to remark that linear temperature–moisture interaction was significant at a level of probability of 10% (calculated  $F = 2.91$ , tabulated  $F = 2.73$  for  $\alpha = 0.10$ ). The no significance of the interaction at the original level of probability ( $\alpha = 0.05$ ) was partially associated with the high variation due to experimental error. In fact, 57% of the total sum of squares was

related with factors other than temperature and moisture.

To analyse the spatial variation of the response of N mineralization, an ANOVA was calculated for each sampling. As no replicates existed within the sampling sites, the  $F$ -values may be calculated by using the mean square of the interaction (Neter and Wasserman, 1974). By doing this, only the individual effects of the factors may be analysed. Significant effects ( $P < 0.05$ ) were found in only 14 sites for temperature and three for soil moisture, which agrees with the general pattern of the response to temperature and moisture. A high spatial variability of the response was observed for both variables; i.e. the response to temperature ranged from  $-0.005$  to  $0.06$  mg kg<sup>-1</sup> d<sup>-1</sup> °C<sup>-1</sup>. These results suggest that other factors varying at the small spatial scale of the sampling sites obscure the response pattern.

#### Models of temperature–moisture dependence

An implicit assumption of equations (7) and (8) is that  $B$  and  $Q_{10}$  are independent of soil moisture. If this is not true, the automatic fit of the experimental data may induce a loss of information concerning the actual effect of moisture and its interaction with temperature. To test this we analysed the response of N mineralization to temperature for each moisture content. Neither  $B$  nor  $Q_{10}$  differed significantly ( $P < 0.05$ ) between moisture contents, implying that both parameters may be assumed as being independent of soil moisture. Therefore, the fit of the data as a whole performed with equations (7) and (8) only differed slightly from the individual fits. The estimated  $B$  and  $Q_{10}$

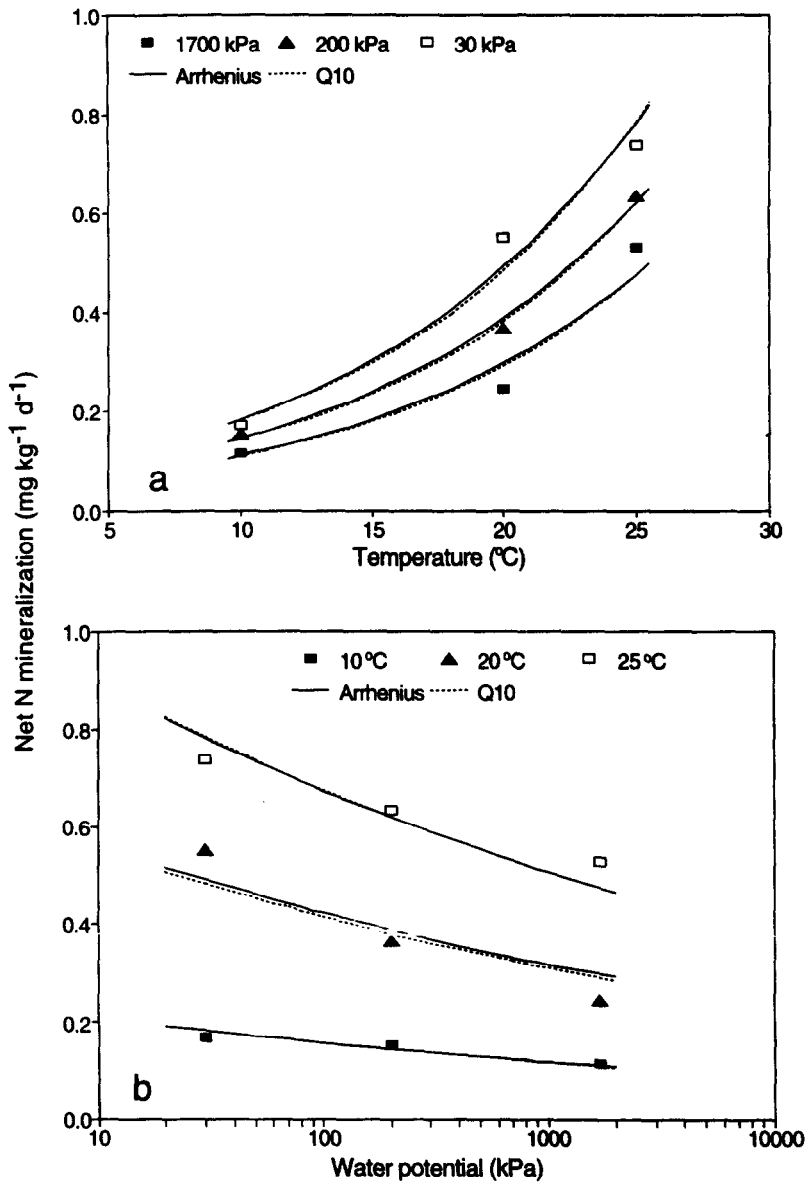


Fig. 3. Relationship between the observed and the fitted rates of N mineralization: (a) as a function of temperature; (b) as a function of soil moisture.

were 8152°K and 2.63, respectively, which are within the range of values reported for N mineralization (Stanford *et al.*, 1973; Addiscott, 1983; Ellert and Bettany, 1992; Grundmann *et al.*, 1995). The Arrhenius and the  $Q_{10}$  functions fitted the data equally well ( $r^2 = 0.972$ ,  $P < 0.05$ ) and were almost fully superposed for all of the levels of temperature and moisture (Fig. 3). The fitted functions were:

$$R_N = 9.1 \times 10^{11} \psi^{-0.124} e^{(-8152/T)} \quad (9)$$

for the Arrhenius function, and

$$R_N = 3.40 \times 10^{-13} \psi^{-0.125} 2.63^{(T/10)} \quad (10)$$

for the  $Q_{10}$  function.

No differences between both functions were observed by Ellert and Bettany (1992), who used them to describe N and S mineralization as a function of temperature (5–30°C). As discussed by these authors, it seems that in spite of the conceptual differences between both models, the response pattern is similar for the range of temperature supporting biological activity. The good fit observed between the experimental data and the models does not necessarily imply the description of the actual mechanisms of N mineralization. Even if the Arrhenius function has a thermodynamic basis, it oversimplifies the mechanisms controlling the response to temperature. Moreover, incubation studies involve simultaneous immobilization and mineralization, and these functions were proposed

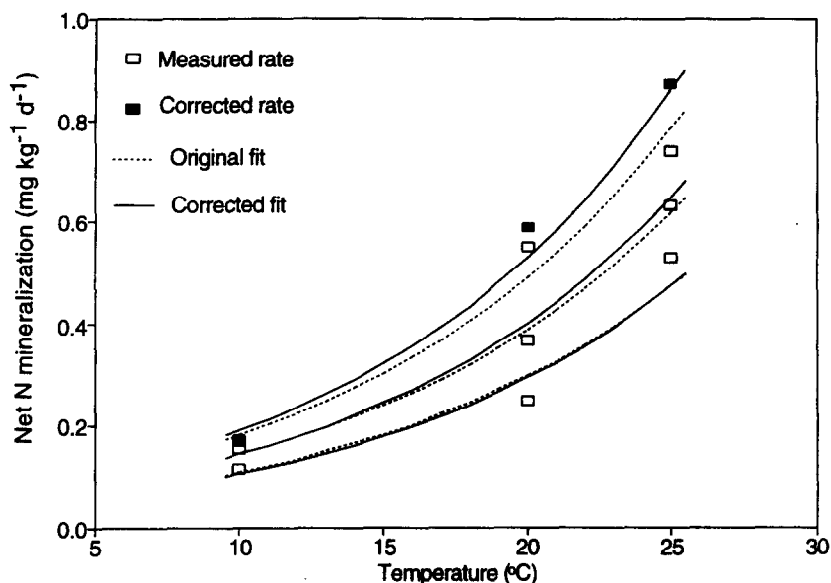


Fig. 4. Comparison of the original fit and the fit corresponding to data corrected to take into account only the aerobic fraction of the soil. Only the fits performed with the model based on the Arrhenius function are shown.

to describe individual process. If the immobilization to mineralization ratio is constant, the Arrhenius and the  $Q_{10}$  functions might well describe net N mineralization in spite of their confounded pattern. There is some evidence that immobilization in undisturbed soil samples increases at low temperature (Frazer *et al.*, 1990) and at high water potential (Mazzarino *et al.*, 1991). Therefore, discrepancies between experimental data of net N mineralization and the Arrhenius or the  $Q_{10}$  functions observed by some authors (e.g. Macduff and White, 1985; Ellert and Bettany, 1992) may be partially due to a differential effect of the environmental factors on immobilization and mineralization.

Figure 3 clearly shows an interaction effect of temperature and moisture (no parallel response): N mineralization was more responsive to a given factor when the level of the other one was more favourable for the process. For example, the response to soil moisture was higher at 20 or 25°C than at 10°C [Fig. 3(b)]. As discussed above, no parameters of the models depended simultaneously on temperature and moisture. Thus, from the two models tested here, the observed interaction was induced by the combined individual effects of these factors. Undoubtedly, this may be a simplification by the models of the numerous mechanisms controlled simultaneously by temperature and soil moisture (e.g. soil aeration).

#### Effect of soil aeration on the response pattern

At 0.280 kg kg<sup>-1</sup> of gravimetric water content the calculated anaerobic fraction was 2% at 10°C, 7% at 20°C and 15% at 25°C. The anaerobic fraction increased with temperature due to the increase of

O<sub>2</sub> consumption by microbial respiration. These results show that denitrification could be one of the possible processes affecting the rate of net N mineralization for the highest water content tested in this study. To examine the effect of soil aeration on the observed response of N mineralization, we corrected the rates measured in the laboratory experiment by using a simple relationship

$$\text{Corrected rate} = \text{measured rate} / (1 - f_a) \quad (11)$$

where  $f_a$  is anaerobic fraction of the soil ( $0 \leq f_a \leq 1$ ). Finally, the set of corrected rates were fitted with the two alternative models. Because the models did not present differences, Fig. 4 only shows the fit made with the model based on the Arrhenius function and the comparison with the original fit of Fig. 3(a). The models described well the corrected data ( $r^2 = 0.961$ ,  $P < 0.05$ ); the recalculated  $B$  (8405°K) and  $Q_{10}$  (2.55) values were slightly different to those obtained for the original fit. Note that the value of gravimetric water content for fully saturated aggregates vary with the bulk and aggregate density. For the same bulk density used in this simulation, and an aggregate density of 1.5 Mg m<sup>-3</sup>, the critical water content is 0.303 kg kg<sup>-1</sup> (WFP 64.5%). In this case, aggregates will be fully aerobic for all of the tested levels of soil moisture.

This analysis indicated that the Arrhenius and the  $Q_{10}$  functions may describe data involving only the direct effect of temperature and, eventually, data with confounded direct and indirect effects. As a consequence, these functions do not provide a full explanation of the response of N mineralization when the aeration status of the soil is unknown. As

a simple approach, information on the bulk density and the aggregate density may be helpful to explain the experimental data and the fit obtained with the Arrhenius and  $Q_{10}$  functions.

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