FACTORS AFFECTING NITROGEN MINERALIZATION UNDER LABORATORY CONDITIONS WITH SOILS FROM A WHEAT-BASED ROTATION TRIAL

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ABSTRACT

Mineralization of organic forms of nitrogen (N) in the field can influence the amount of N available to crops and the response to N fertilizer. The amount and rate of N mineralized in any season is dependent on environmental factors, mainly moisture and temperature. Total N in soils can vary with soil type and also as a result of different cropping systems. A cereal rotation trial initiated in 1986 at the International Center for Agricultural Research in the Dry Areas, (ICARDA) main research station, Tel Hadya, in northern Syria, indicated that the cropping alternatives, especially forage legumes, had induced changes in both soil organic matter and total soil N. In order to assess the potential effect of mineralization of total N on soil mineral N and crop growth, a series of laboratory experiments were conducted to simulate environmental conditions encountered in the field, i.e., the range of both temperature and moisture and alternating wetting and drying that occurs in any rainfed cropping system. In general, optimum mineralization occurred at the higher temperatures (24–30ºC) and moisture content of 100% field capacity. Repeated wetting and drying of soil samples under constant temperature enhanced mineralization. There were substantial differences between rotations, with soil from the medic rotation consistently having the highest mineralization rate. These laboratory observations corroborated data from the field trial reflecting increased soil mineral N and crop N content and reduced crop response to applied N.

Keywords : arid region, wheat - based rotation, legumes, *in-vitro* nitrogen mineralization, environmental conditions, temperature, wetting-drying cycles, moisture content

INTRODUCTION

The Middle East region, despite being the center of origin of many of the world's major crops and the cradle of Western civilization, is now a food-deficient region. While drought is the main crop-limiting factor due to low and erratic rainfall and high temperatures, adequate nutrition through commercial fertilizer use is vital to obtaining viable economic yields (Ryan and Matar, 1992; Ryan, 1997). Without fertilizers, nitrogen (N) deficiency is ubiquitous for all crops except N-fixing legumes. With increasing fertilizer costs, concern has centered on efficiency of N fertilizer use and on cropping systems that enhance N use from legumes (Harris, 1995). In view of concerns about sustainability of natural resource use (Jones, 1993) and the emerging concept of soil quality (Ryan, 1998), crop rotations, *i.e*., growing different crops in a defined sequence, have come back into vogue.

While cereals have been alternated with fallow in the Middle East region from time immemorial as a strategy to mitigate the effects of drought, food and feed legumes are grown in rotation with cereals in more favorable areas. Because of land-use pressure, fallow is disappearing in all but the driest areas and is being replaced by continuous cereals (Diaz-Ambrona and Miniguez, 2001). As livestock-raising has traditionally been part of the system, feed has been provided by stubble and annual or perennial forages (Cooper *et al*., 1987).

Thus, long-term trials established in the mid-1980s at ICARDA were designed to find a viable alternative to fallow and continuous wheat, and at the same time provide feed for the animal sector as well as a cash crop for farm income (Harris, 1995). Early observations had indicated that legumes in rotation with wheat seemed an alternative to continuous wheat and fallow (Ryan and Pala, 1999; Harris *et al.,* 1995). Some forage legumes (vetch, medics) not only provided animal grazing during the non-cereal phase, but also increased the soil's content of both total and mineral N. Details of the trial have been described by Harris *et al.* (1995).

In brief, the trial, initiated in 1986, involved durum wheat (*Triticum durum,* var *durum*) grown in rotation with fallow; summer crop, *i.e.,* watermelon (*Citrullus vulgaris*), if residual moisture was adequate; chickpea (*Cicer arietinum*); lentil (*Lens culinaris*); vetch (*Vicia sativa*) and medic (*Medicago*) pasture; and wheat (i.e., continuous). Auxiliary treatments included variable grazing intensity of the cereal stubble by sheep (no grazing, medium, high) and four levels of N applied to the cereal phase (0, 30, 60, 90 kg ha^{-1}). The treatments were assessed in terms of biological yield and animal offtake, in addition to soil moisture and both physical and chemical properties. As the trial progressed, it became evident that the alternative rotations have a variable effect on total and mineral soil N as well as organic matter (Ryan *et al.,* 1992).

Though the bulk of the soil N from biological fixation by legumes is immobilized in organic N forms, the extent to which the pool becomes available to the succeeding crop depends on the process of mineralization (Cabrera and Kissel, l994). The extent of N release in any season depends on the combined and interactive effects of temperature and moisture (Stanford and Epstein, 1974; Stanford *et al*., 1974,1975), as well as the extent of wetting and drying (Van Schreven, 1968), a common feature of drought-prone rainfed agriculture in the Middle East region. In assessing the impact of rotations on soil quality, particularly N reserves, it is important to estimate the extent to which such reserves are likely to be mineralized under field–simulated conditions, *e.g.*, the mineralization ability or potential (Matar *et al.,* 1991). Therefore, the objective of this study was to establish if field observations from a long-term rotation trial could be corroborated and predicted by laboratory mineralization data.

MATERIALS AND METHODS

Soil Properties

The soil used in the study was taken from the site of the “Cropping Systems Productivity” trial at ICARDA’s main station, Tel Hadya, near Aleppo in northern Syria. Details of the trial have been described by Harris *et al.* (1995). As the trial progressed, it became evident that the alternative rotations have a variable effect on total and mineral soil N as well as organic matter (Ryan *et al.,* 1992). While both organic matter and total N showed similar variation with rotations, with a relatively constant C/N ratio, lowest values were from the fallow and watermelon - summer crop - rotation (essentially fallow), and the highest values with the medic and vetch rotations (Table 1).

TABLE 1
Mean Organic Matter and Total Nitrogen with Rotation of Wheat after 8 Years Rotation with Alternative Crops

Property	Wheat	Fallow	Watermelon	Lentil	Chickpea	Vetch	Medic
Soil organic matter, %	1.14	1.12	1.06	1.18	1.20	1.19	1.39
Total nitrogen, mg kg^{-1}	768	729	719	790	764	813	903
C: N ratio	8.6	8.6	8.4	8.7	8.8	8.9	8.7

The soil was classified as a fine clay, montmorillonitic, thermic, *Chromic Calcixerert* (Ryan *et al.,* 1997). It had a 65% clay and 32% silt, a pH of 8 and 24.6% $CaCO_3$. Because of the swelling/shrinking capacity of the soil, bulk density throughout the profile changes with the occurence of dry and wet periods in the season. At field capacity the moisture content is 42% and at the permanent wilting point it decreases to 26%.

Experimental Procedure

For this study, soils were taken in June 1995 after harvest, from plots receiving no additional treatment : zero stubble grazing and zero N fertilizer. This was important in order to separate the impact of the alternative crop, its residus from the other effects leading to N build-up and turnover. Although both phases of the rotation are present every year, soils were sampled from the "Legume phase". These were fallow (1) ; summer crop, i.e., watermelon, if residual moisture was adequate (2) ; chickpea (3) ; lentil (4) ; vetch (5) ; medic (6); and wheat

(7). Each sample was a composite of three sub-samples per plot at 0–20 cm depth. After crushing with a wooden mallet, the soil was put through a 2-mm sieve. Soil samples were air-dried. Separate experiments were set up in the laboratory to assess the influence of environmental factors in a simulation of field conditions.

1. Temperature

Air-dry soil (20 g) in duplicate was mixed with 40 g silica sand, previously cleaned by distilled water and dried. The mixture was transferred to 60-mL plastic syringes serving as leaching tubes, which had a piece of glass wool at the bottom to eliminate soil loss during leaching. Then 25 mL of a dilute nutrient solution (1 *mM* $CaCl_2$, 1*mM* $MgSO_4$, 1 *mM* KH_2PO_4) was added to each tube. Each syringe was covered by a piece of glass wool and aluminium foil to prevent drying. The tubes were then incubated at about 8, 24, and 30ºC. Mineralized N was recovered on a weekly basis for 6 weeks (in addition to time 0) by leaching with 75 mL of the dilute nutrient solution in increments of 25 mL. Leachates were then analyzed for nitrate, nitrite, and ammonium (Bremner and Keeney, 1965). After each leaching, the water content of the tubes was standardized by applying suction at 0.25 bar.

The study of the mineralization pattern was approached by finding the slopes of the equation between the cumulative N against $t^{1/2}$. These relationships were found linear for a short incubation period. Also, slopes multiplied by a constant (6.5) gave the mineralization potential (Stanford *et al.*, 1974). Statistical analysis of mineralized N under different rotations and at the three temperatures was conducted using a non-parametric two-factorial analysis of variance (Krushkal-Wallis test). Slopes of rotations at each temperature were compared between them (Bailey, 1981).

2. Moisture

Soil samples (50 g), in duplicate, representing the seven rotations were placed in glass petri dishes and moisture content was adjusted to 50, 75, 100, and 150% of field capacity. These were then incubated at 30ºC for 14 days. Subsequently, the samples were extracted with 2 *M* KCl by shaking for 1 hour followed by filtration.

3. Wetting-Drying Cycles

Soil samples (50 g) were placed in petri dishes, wetted to a pre-determined field capacity (100%) and then subjected to six successive wetting and drying cycles; a complete batch representing all treatments was removed after each cycle which consisted of covering the petri dishes for 2 days and uncovering them at room temperature (24ºC) for another 2 days. On Day 5 of each cycle, the soil samples were extracted with 100 ml 2 *M* KCl for determination of mineral N forms.

RESULTS

The cumulative amount of N mineralized in the 6-week incubation period was influenced by temperature and the rotation (Table 2). All values increased with temperature, with differences between 8 and 24ºC being highly significant based on a Kruskal-Wallis test. With further increase in temperature to 30ºC, all rotations produced higher amounts of N, except the continuous wheat rotation. Mineral N values from some rotations, i.e., vetch and medic, almost doubled between 24 and 30ºC. At these temperatures, the highest amounts of N mineralized came from these rotations. At the highest temperature, 9.12% of the total N for medic and 5.70% of that for vetch was mineralized.

The patterns of N mineralization varied through the 6-week incubation period, as illustrated for the two different rotations : fallow and medic (Fig. 1). Mineralization rate varied with the temperature. At the lower temperatures, proportionally more of the N from fallow was released in the first 2 weeks. This flush of mineralization was observed for 4 other rotations. This could be possibly linked to the easily degradable organic N, as mineral N present in the soil prior to incubation was determined by leaching at week 0. During seasons when the summer crop was not grown, initial mineral N could be highest in late summer in the summer crop (2), among legumes medic (6) had the highest values (Matar and Harris, 1990). These concentrations varied between 12 mg/kg soil (Treatment 2) and 6 mg/kg soil (Treatments 3 and 7). In this study, mineral N was smaller than 10 mg/kg soil.

However, with the medic rotation, there was a peak after 3 weeks regardless of incubation temperature, with values dropping off substantially after that. This peak at week 3 was common to 11 treatments out of 14 for the temperatures 24 and 30 ºC. The ability of the residues to mineralize was studied using the slopes of the equations (Mineral N *vs* $t^{1/2}$). Medic had invariably the largest slope, followed by Vetch and chickpea among the legumes. Summer crop, fallow and Lentil had the smallest values (Table 2). The result of Lentil could be associated with the mode of hand harvest of the crop, which could lead to an important removal of the residues including possibly some of the roots and the nodules. This could seem contradictory with the field results, as after the legume phase, total soil N had the following succession: Medic>Lentil>Vetch>Chickpea=Fallow=Wheat>Summer crop (Ryan *et al.,* 1999). But, mineral N determined under field conditions, was the highest after medic and the lowest after wheat/wheat or wheat/lentil. This could be explained by the fact that residues built in the lentil treatment were not easily decomposed and could contribute more to the less-soluble fraction.

One common feature was the fact that virtually all the mineral N was in the nitrate form, with only little amounts of ammonium-N detected (around 10% of mineral N). This clearly indicates that the ammonium mineralized from organic N was rapidly transformed to nitrate.

TABLE 2
Cumulative N and Corresponding Percentages of Total N Mineralized in Soils from Seven Rotations Incubated up to 6 Weeks at Various Temperatures

Rotation	8ºC			24ºC			30ºC		
	Slope	mg kg^{-1}	%N	Slope	mg kg^{-1}	%N	Slope	mg kg^{-1}	%N
Lentil	4.67b	9.9	1.31	10.71cd	15.7	2.08	12.52d	19.1	2.54
Watermelon	2.64c	5.3	0.78	8.59d	16.7	2.44	16.23cd	23.4	3.42
Vetch	4.72b	9.3	1.20	14.03c	23.0	2.97	30.64ab	44.3	5.70
Fallow	3.32bc	7.5	1.04	9.01d	17.3	2.41	21.93c	27.9	3.89
Chickpea	5.03b	7.3	0.97	21.62b	31.0	4.13	29.64b	42.3	5.64
Wheat	6.52ab	13.2	1.81	28.24ab	45.0	6.18	13.01d	*22.2*	*3.45*
Medic	9.27a	12.7	1.49	31.40a	46.1	5.39	53.32a	77.7	9.12

Within each column, slopes followed by the same letter are not significantly different at $p<0.05$.

Calculated mineralization potentials based on the slopes at different incubation temperatures clearly showed the capacity of the medic rotation to supply mineral N to the succeeding crop, with continuous wheat, chickpea, and vetch being intermediate, and a low potential of fallow and summer crop, and least for lentil (Fig. 2). These potentials could only be used as an indication of the readiness for mineralization and the availability of mineral N. The data on N mineralization with varying moisture content clearly showed a maximum at 100% field capacity, with a consistent increase with moisture (Table 3). Where the moisture content exceeded field capacity by 50%, there was a sharp decline in mineralized N, presumably due to the anaerobic conditions reducing nitrification, as mineralization is an aerobic process. While there were differences between rotations with varying soil moisture, the patterns were not consistent with the rotation effect under varying incubation temperatures. However, values from the medic rotation were consistently highest and those from the lentil rotation were consistently lowest. The latter is consistent with the results obtained from the incubation at 30 C, as lentil gave one of the smallest slopes.

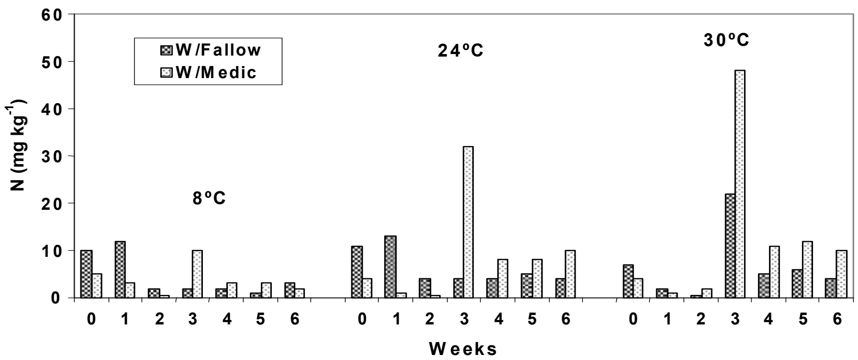

Figure 1. Mineral nitrogen in soils from fallow and medic rotations incubated at three temperatures.

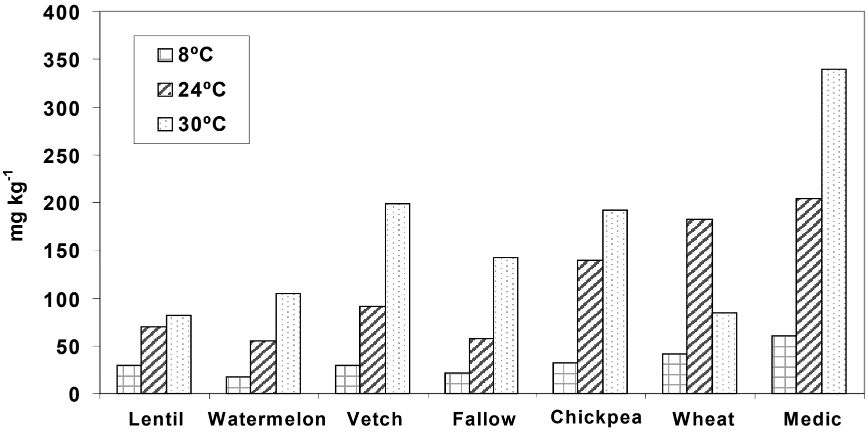

Figure 2. Nitrogen mineralization potentials of soils from seven crop sequences at three temperatures.

The process of wetting and drying consistently increased the amount of N mineralized with each cycle (Table 4). All samples regardless of the number of cycles of wetting–drying were subject to the same incubation time and temperature. Again, as with temperature and moisture effects, there were consistent differences due to rotation, with the medic rotation being generally highest. For this treatment, moisture content at the end of each cycle fluctuated between 10.5% at its highest and 7.76% at the lowest (oven-dry weight basis)

TABLE 3
Mineral N in Soils from Seven rotations under Constant Temperature (24ºC) and Different Moisture Levels for 14 Days

Rotation	Moisture Level (% Field Capacity)			
	50	75	100	150
	---------------------mg kg^{-1}------------------			
Lentil	2.8	2.7	6.2	10.2
Watermelon	9.5	12.2	18.1	12.1
Vetch	7.1	9.3	18.0	13.1
Fallow	12.2	14.4	20.2	14.8
Chickpea	8.8	8.8	17.0	10.9
Wheat	6.7	10.6	14.6	11.9
Medic	11.5	14.7	26.7	11.9

TABLE 4
Mineral N in Soil from Seven Rotations under Different Wetting and Drying Cycles

Rotation	Wetting-Drying Cycles					
	1	2	3	4	5	6
	--------------------------------mg kg^{-1}--------------------------------					
Lentil	3.1	8.1	7.5	10.8	13.7	19.6
Watermelon	8.8	10.5	11.2	15.3	17.0	41.0
Vetch	5.1	9.6	8.6	10.6	11.6	20.1
Fallow	9.0	7.4	9.4	14.6	15.5	23.4
Chickpea	5.7	8.7	12.9	14.9	14.4	24.1
Wheat	6.5	8.3	8.1	11.9	12.1	22.8
Medic	8.6	14.9	16.2	23.4	21.0	34.6

DISCUSSION

The data presented in this incubation study clearly showed the importance of environmental factors such as moisture and temperature and their interactions on the process of N mineralization. These factors influence the growth of soil microorganisms that are responsible for breakdown of organic matter, particularly organic N forms, which vary with the nature of the organic matter, which in turn is dependent on the rotation. These results are consistent with other findings that temperatures in the order of 25–35ºC are optimum for mineralization (Honeycutt and Metcalf, 1994). Similarly, mineralization is promoted where the soil is relatively moist (Linn and Doran, 1984), but the process is retarded where field capacity is exceeded.

Our data, too, showed that repeated wetting and drying stimulates more mineralization than continuously moist conditions (Cabrera and Kissel, 1994). What is new in our study is the high mineralization potential (of the organic N) from the medic rotation. This reflected not only the total N supply but probably the nature of the organic N, with a relatively higher proportion of the N forms presumably being relatively soluble. The increased N reserves from medic, which originated in biological N fixation, were probably due to the crop root system and leaf-fall, which is subsequently incorporated by tillage. Under field conditions, mineral N in the top 100 cm was highest for medic then chickpea then fallow. After the legume phase, fallow had the highest value then medic and chickpea. For total soil N contents, medic also had the largest values in both the wheat and legume phases (Ryan *et al.,* 1999). The low values due to lentil were probably because this short-growing crop is harvested by pulling from the roots, leaving little mineralizable N.

As with all incubation studies, they are only meaningful if they reflect soil behavior under field conditions. Notwithstanding the discrepancies between both sets of conditions, the laboratory indices confirmed observations from the field trial that rotations had a different effect on total mineral N as reflected by analyses at the end of the cropping season (Harris *et* *al.,* 1995). Thus, under field conditions, mineral N was highest from the medic and least for continuous wheat, fallow, and lentil. Concentrations of N in grain and straw reflected the increased soil N supply (Ryan *et al.,* 1992).

Throughout the growing season, soil N was mineralized to an extent depending on temperature and moisture conditions. While little or no mineralization occurs when soil temperatures are low *e.g*., < 10ºC in December – February, with a subsequent rise in temperature and rapid crop growth in early spring in typical Mediterranean rainfed conditions (> 20ºC), mineralization would also proceed rapidly. As rainfall is usually intermittent in most seasons, mineralization would also be intermittent. During cyclic dry spells which limit growth, conditions for soil mineralization would also be limited. Thus, there is synchronization between what occurs above and below ground.

The goal of crop management for conditions in the Middle East region is to obtain adequate yields and efficiency of inputs such as N. Legume–based rotations are seen as important in system sustainability and improving soil quality. While this trial is now terminated, it is likely that the rotation effects would become accentuated with time and that a legume-based rotation would eventually be self-sustainable in terms of N supply (Ryan and Pala, 1999). Mineralization studies that encompass realistic field conditions can serve as indicators of N *availability and system sustainability.*

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