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CHANGES IN MINERAL AND MINERALIZABLE N OF SOIL INCUBATED AT VARYING SALINITY, MOISTURE AND TEMPERATURE REGIMES

ASMA LODHI[1*], M. ARSHAD[2], F. AZAM[3] AND M.H. SAJJAD[1]

[1]Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan
2Department of Botany, University of Agriculture, Faisalabad, Pakistan
[3] Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan.

Abstract

A laboratory incubation experiment was conducted to study the effect of factors like moisture, salinity and temperature on the release of N in plant-available forms (NH_4 and NO_3+NO_2-N) and potentially mineralizable N in soil over a period of 8 weeks following amendment with leguminous plant residues. In this experiment, soil samples salinized to EC_e 7, 9, and 18 dS m^{-1} (original EC_e was 5.0 dS m^{-1}) were amended with 0.5% plant material of *Sesbania aculeata* and incubated at three moisture levels of 15, 30 and 45%, w/w and three temperature regimes of 20, 30 and 40°C for 8 weeks. Soil samples were sacrificed at 2, 4 and 8 weeks for the determination of NH_4-N, NO_3+NO_2-N and mineralizable N. Ammonification of organic N as determined by the accumulation of NH_4-N in soil was found to increase with time as salinity, moisture and temperature increased. However, the increase was more pronounced at higher moisture levels. While temperature had a positive effect on nitrification, increased salinity and moisture depressed the process. Net mineralization of N increased with time in all the treatments; the process being enhanced at higher incubation temperature with a maximum at 40°C. Salinity and high moisture had a depressing effect on the mineralization of N.

The content of mineralizable N determined by NH_4-N accumulation following 2 weeks of incubation under submerged conditions in soil remained higher under high moisture conditions, while high salinity and temperature had a variable and negative effect. Apparently, high moisture content conserved organic N due to reduced mineralization, while high temperature had an opposite effect. A complete loss of NO_3-N was observed during incubation of soil samples for the determination of mineralizable N. This was attributable to denitrification as sufficient amount of easily oxidizable C was still present in the soil after 8 weeks of incubation under relatively aerobic conditions.

Introduction

Salt-affected soils are characterized by high concentrations of soluble salts and low organic matter and nitrogen content (Ashraf & Rehman, 1999). It is a common knowledge that supply of N limits crop growth and productivity more often than the supply of any other nutrient element. Under saline conditions, the role of N becomes more important as the biological activity is poor and hence any possibility of N addition through biological fixation is meager. As a result, application of N fertilizer will significantly increase crop yield on salt-affected soils (Aslam *et al*., 1994; Azam *et al*., 1992a, b; Botella *et al*., 1993; Miah and Panaullah 1991; Shaviv and Hagin 1992; Shen *et al*., 1994; Steppuhn *et al*., 1994). However, because of low organic matter level of such soils, conservation of N through microbial immobilization remains limited. As a result, fertilizer N economy of salt-affected soils may not be comparable to normal agricultural soils.

[*]E-mail: alv51062@ yahoo.com

A workable approach for the economic utilization of salt affected soils for crop production vis-à-vis N availability is the use of leguminous green manures (Sandhu & Malik, 1975). This practice not only improves the physico-chemical and biological properties of the soils, but helps improve the N availability status as such or through enhanced N mineralization from soil organic matter (Azam, 1990; Woods *et al*., 1987). However, the process of N mineralization is significantly affected by factors that affect soil microflora and their functions. Excessive salts, soil temperature and moisture are some of the important factors affecting these processes (Ashraf & Rehman, 1999; Marschner, 1991). While considerable information is available on the effect of these factors on N mineralization i.e., ammonification and nitrification (Sethi *et al*., 1993; Westerman and Tucker, 1974), hardly any studies have been reported on the dynamics of mineralizable N. Therefore, the studies reported here were aimed at understanding the effect of three important environmental factors viz., moisture, salinity and temperature on i) mineralization of N and its accumulation as NH_4 and NO_3+NO_2-N in soil and ii) changes in potentially mineralizable N during long term incubation. These studies were undertaken after incorporation in soil of powdered plant material of *Sesbania aculeata* which is a commonly used green manuring leguminous crop.

Material and methods

Soil: Soil used in this study was collected from the fields of Post-graduate Agriculture Research Station of the University of Agriculture, Faisalabad. Air-dried and sieved (<2 mm) soil had the following characteristics: organic C, 0.6%; total N, 0.055%; mineral N (NH_4+NO_3+NO_2), 25mg kg^{-1} soil; electrical conductivity (EC; 1:1; soil:water suspension), 5 dS m^{-1}; pH (1:1; soil:water suspension), 7.9; sand, 39.6%; silt, 26.2%; clay, 34.2%; and water holding capacity, 30%. Relatively high organic matter and N content is uncharacteristic of salt-affected soils and was due to the previous cropping history that involved cultivation of *Sesbania*. Organic C was determined by microwave-colorimetric method (Azam & Sajjad, 2005), total N by micro-kjeldahl method (Bremner, 1996) and mineral N by steam distillation method (Keeney & Nelson, 1982). Potentially mineralizable N was determined as described by (Stanford & Smith, 1972). Briefly, 20-g portions of the soil were incubated with 20 ml water (submerged conditions) for 2 weeks at 30°C. The soil-water suspension was quantitatively transferred to flasks using 2*N* KCl for rinsing and distillation using MgO & Devarda's alloy to separately determine NH_4 and NO_3+NO_2-N content (Keeney & Nelson, 1982. Analyses for the parameters (EC, pH and textural composition) were performed as described in USDA Handbook 60 (Richards, 1954).

Artificial salinization of soil: Portions of soil were treated with solution of $CaCl_2$, $MgSO_4$, NaCl and Na_2SO_4 mixed in the ratio of 2:1:3:4 so as to obtain EC levels of 7, 9, and 18 dS m^{-1}. For this purpose, appropriate volume of the solution was applied to the required amount of soil and the material was left to dry. After drying, the soil samples were ground to pass through a 2-mm screen and portions analyzed for EC.

Incubation experiment: Hundred-g portions of the soil were placed in 200-ml capacity plastic bottles and amended with 0.5% of finely powdered plant material of *Sesbania aculeata*. The amended soil samples were incubated under different conditions of temperature (20, 30 and 40°C) and moistures (50, 100 and 150% of water holding capacity i.e., 15, 30 and 45% v/w). Enough replicates were incubated to enable withdrawal of 3 bottles at 0, 2, 4 and 8 weeks of incubation (total bottles, 324). The withdrawn samples were immediately frozen and then freeze dried. Portions of freeze-dried samples were analyzed for i) NH_4^+ -N, ii) NO_3+NO_2-N and iii) potentially mineralizable N as referred to above.

Statistical analyses: The data were subjected to appropriate statistics using the computer software MS-EXCEL. Since the studies were conducted under controlled laboratory conditions, treatment differences were judged mainly on the basis of standard error and coefficient of variance. At places, however, relationship between different parameters was drawn using coefficient of correlations.

Results

Changes in mineral N (NH_4 and NO_3) content of the soil: Figure 1 shows the data on general trends for NH_4-N accumulated in soil incubated for 2-8 weeks under varying levels of salinity, temperature and moisture. The amount of NH_4-N accumulated varied from 0 to 27 µg g^{-1} soil under different conditions; the highest values being obtained at 40°C. However, a significant correlation was obtained between NH_4-N accumulated at the three temperature regimes; coefficient of correlations i.e., *r*, being 0.75, 0.81 and 0.83 for 20 *vs* 30, 30 *vs* 40 and 20 *vs* 40°C, respectively (n = 36). As a whole, the amount of NH_4-N increased with the time of incubation under different temperature and moisture regimes, but the trends were not very clear for the effect of salinity. Soil moisture had a much more pronounced effect on the accumulation of NH_4 i.e., the latter increased significantly with increase in moisture content. An increase was also observed with increase in temperature, but the extent was less than that observed for moisture. An increase in NH_4-N accumulation was observed with increase in salinity only up to 4 weeks and hence the average values did not show clear trends.

The content of NO_3-N varied between 0 and 60 µg g^{-1} soil under different conditions; the highest values being obtained at 40°C as was the case for NH_4-N (Fig. 2). Correlations were still better for NO_3-N as compared to those obtained for NH_4-N i.e., the values of *r* were 0.99, 0.97 and 0.98 for the three temperature combinations, respectively. As expected, the amount of NO_3-N increased with time of incubation under all conditions; maximum values being recorded at the highest temperature i.e., 40°C. The amount of NO_3-N decreased with salinity under different temperature and moisture regimes. The process of nitrification increased with temperature, the average being similar at 30 and 40°C. The process of nitrification was stopped almost completely at 45% moisture, while it was substantially inhibited at 30% moisture. The retardation/inhibition of nitrification was apparent from the very onset of incubation i.e., significantly lower amounts of NO_3-N were determined after 2 weeks of incubation at 30 and 40% moisture. The accumulation of NO_3-N increased with time of incubation and was particularly higher after 8 weeks of incubation under all the test conditions.

Cumulative values of NH_4- and NO_3-N in soil at different incubation intervals and under different conditions are presented in Fig. 3. The amount of NH_4 + NO_3-N in soil varied between 0 and 52 µg g^{-1} soil under different conditions; the highest values being obtained at 40°C and at the lowest moisture and salinity regime. The pattern of NH_4 + NO_3-N accumulation under different conditions of salinity and moisture was essentially similar as a highly significant correlation was obtained between the data values for any two temperature regimes; coefficients of correlation being 0.88, 0.91 and 0.88 for 20 *vs* 30, 30 *vs* 40 and 20 *vs* 40°C, respectively. These results suggested that the effect of temperature on total mineral N was comparable under different salinity and moisture regimes. As regards individual factors (taking average for the other factors), mineral N increased with temperature, the increase being significantly higher at 30 and 40°C after 2 weeks of incubation. This effect diminished with time and after 8 weeks, amounts of mineral N were almost comparable at 30 and 40°C. The average values after 8 weeks were, however, higher for the two higher temperature regimes. Although mineral N content of the soil increased with time at different moisture regimes, it was almost similar at 30 and 45% moisture, but significantly lower than that observed at a moisture content of 15% when average values for factors other than moisture were computed. Increasing soil salinity had a consistent negative effect on the accumulation of mineral N at all incubation intervals, although an increase with time was obvious.

Changes in mineralizable N content of the soil: Figure 4 compares at a glance the effect of all the test factors on mineralizable N. A higher content of mineralizable N was apparent at 20°C at the 2 lower levels of salinity. At the two higher salinity levels, however, more mineralizable N was determined at 30 and 40°C as compared to that at 20°C. The effect of moisture was variable although relatively higher values of mineralizable N were obtained at 45% moisture in most cases. Soil salinity had generally a positive effect on mineralizable N. In contrast to the data presented in Figures 1, 3 and 5, mineralizable N was not affected similarly at the three temperature regimes as the coefficients of correlation were either negative or non-significant when data sets for any two-temperature regimes were compared. This is because of the pre-incubation of soil samples for 0-8 weeks under different conditions and the resultant changes in mineralization of N. Taking individual factors (average for the remaining factors), no clear trends were obtained as far as the effect of temperature on mineralizable N is concerned. The effect varied widely with time as well as the temperature regime. On an average, higher amounts of mineralizable N were observed at 20 and 40°C as compared to that at 30°C. The effect of moisture on mineralizable N was relatively more obvious and the latter increased consistently with the level of moisture; the highest amounts being observed at 45% moisture and the lowest at 15%. The effect of salinity was also variable at different incubation intervals although on an average lesser amounts of mineralizable N were obtained under saline conditions i.e., at salinity levels of 7, 9 and 18 dS m^{-1}.

A net and complete loss of NO_3-N that accumulated in soil samples incubated under aerobic conditions (0-8 weeks) was observed in soils subjected to anaerobic conditions (Fig. 5). Such losses are not unexpected especially from soil containing sufficient quantities of easily oxidizable organic matter as in the present study where 0.5% *Sesbania* material was added before incubation under aerobic conditions. Trends in the loss of NO_3-N under different conditions were similar at the three temperature regimes as the coefficients of correlation were highly significant i.e., *r* values were 0.87, 0.89 and 0.94 when computing the data for 20 *vs* 30°C, 30 *vs* 40°C and 20 *vs* 40°C, respectively. Under some of the incubation conditions (especially high moisture content during aerobic conditions), no losses of NO_3-N seemed to have occurred. In fact, these soil samples did not contain any NO_3 –N at the start of anaerobic conditions. Taking different factors separately (average of the remaining two factors), the losses were more at higher temperature and significantly low at high moisture while the level of soil salinity did not show specific trends.

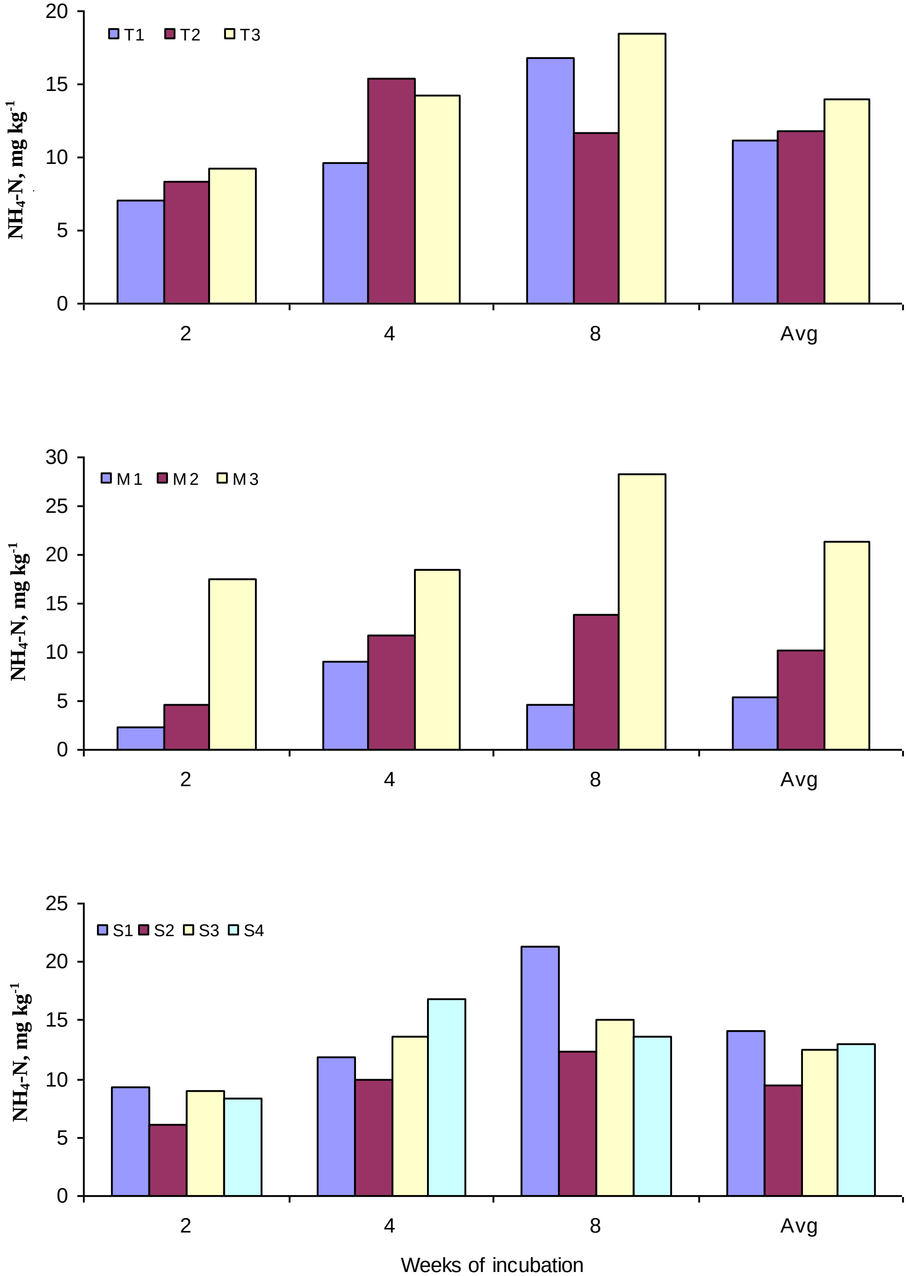

Fig. 1. Changes in NH_4-N content of soils for 8 weeks at different temperature and moisture regimes. T_1-T_3, temperature regimes of 25, 30 and 40°C, respectively; M1-M3, moisture regimes of 15, 30 and 45%, respectively; S1-S4, salinity levels of 5, 7, 9 and 18 dSm^{-1}, respectively.

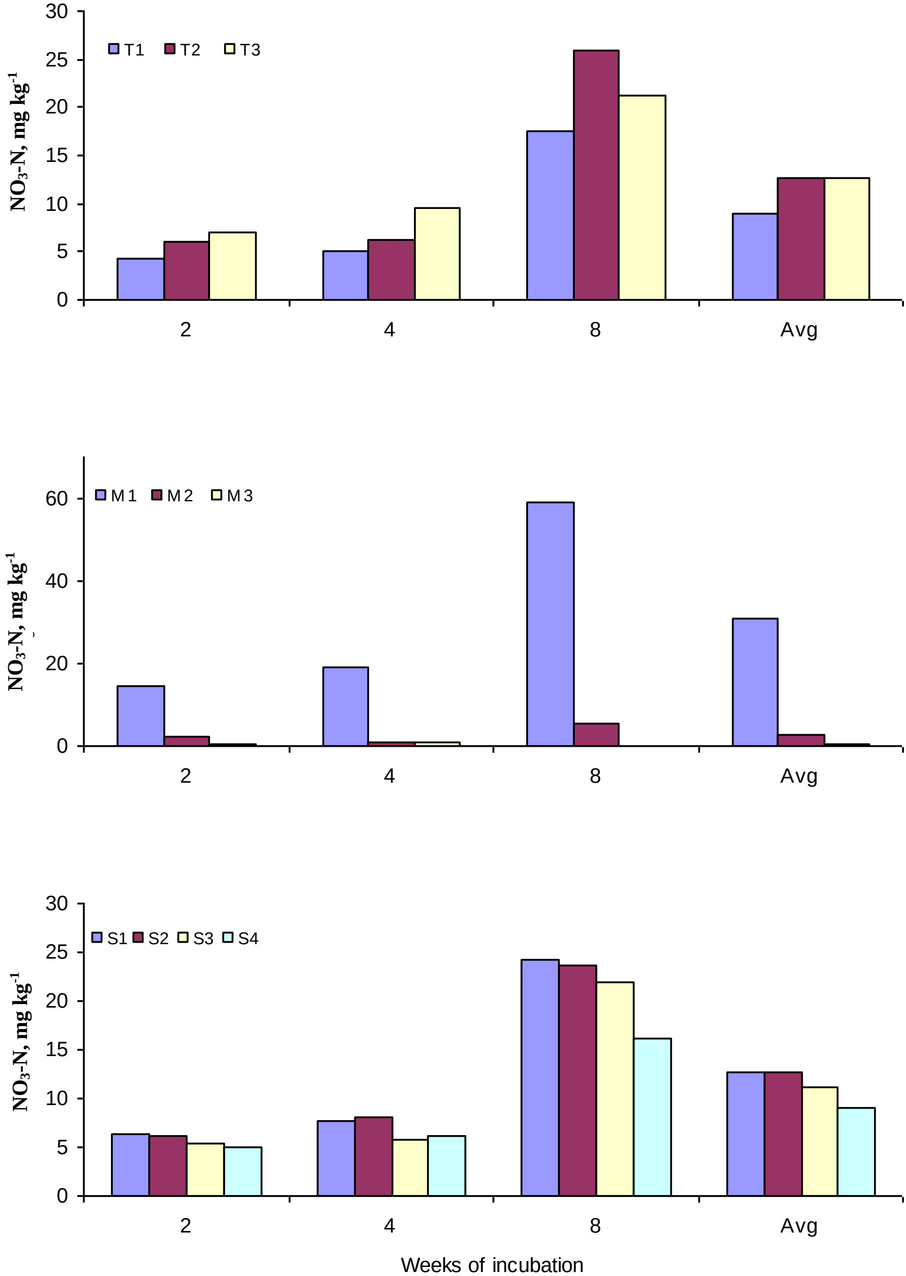

Fig. 2. Changes in NO_3-N content of soils for 8 weeks at different temperature and moisture regimes. T_1-T_3, temperature regimes of 25, 30 and 40^{o}C, respectively; M1-M3, moisture regimes of 15, 30 and 45%, respectively; S1-S4, salinity levels of 5, 7, 9 and 18 dSm^{-1}, respectively.

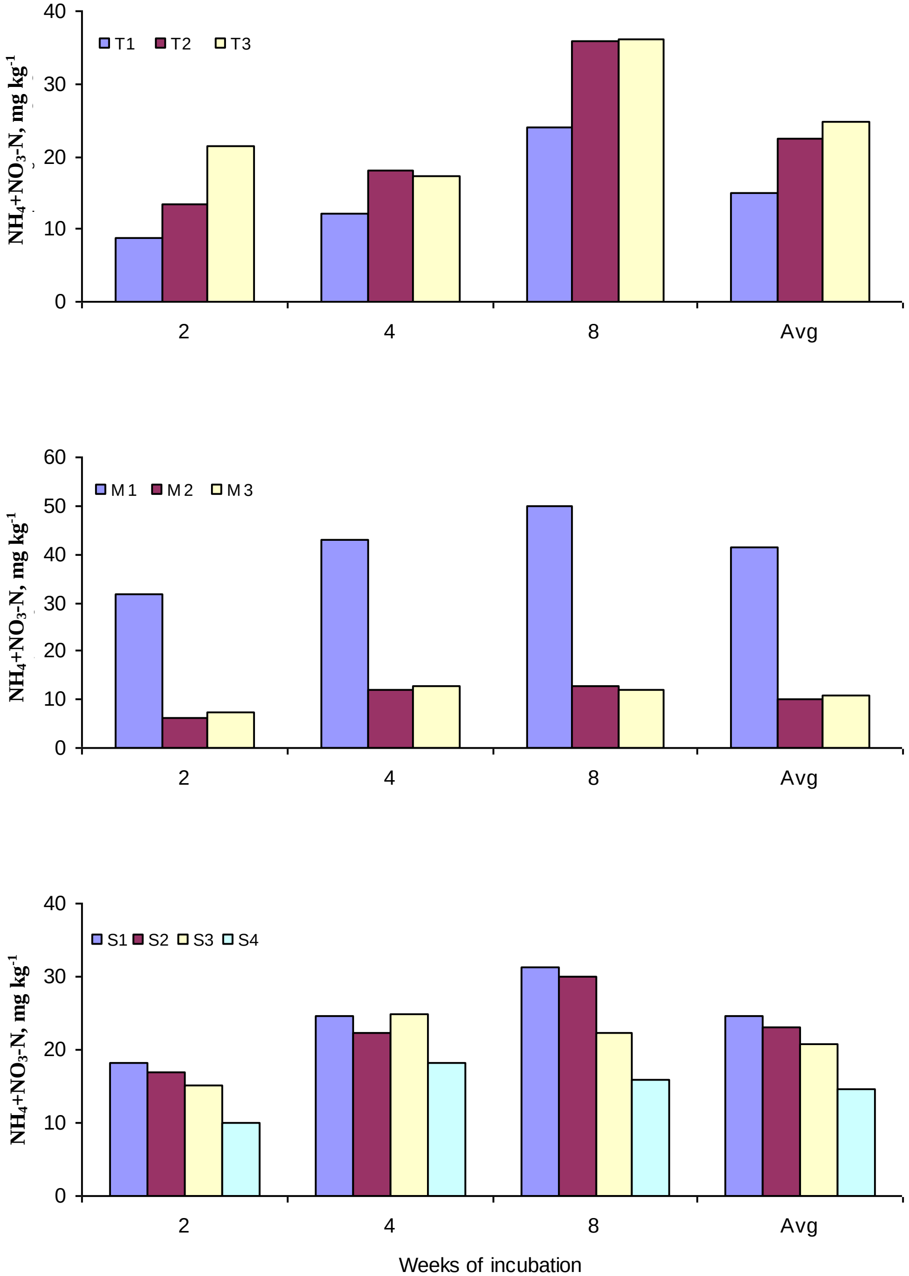

Fig. 3. Changes in total mineral N (NH_4+NO_3-N) content of soils for 8 weeks at different temperature and moisture regimes.

T_1-T_3, temperature regimes of 25, 30 and 40^{o}C, respectively; M1-M3, moisture regimes of 15, 30 and 45%, respectively; S1-S4, salinity levels of 5, 7, 9 and 18 dSm^{-1}, respectively.

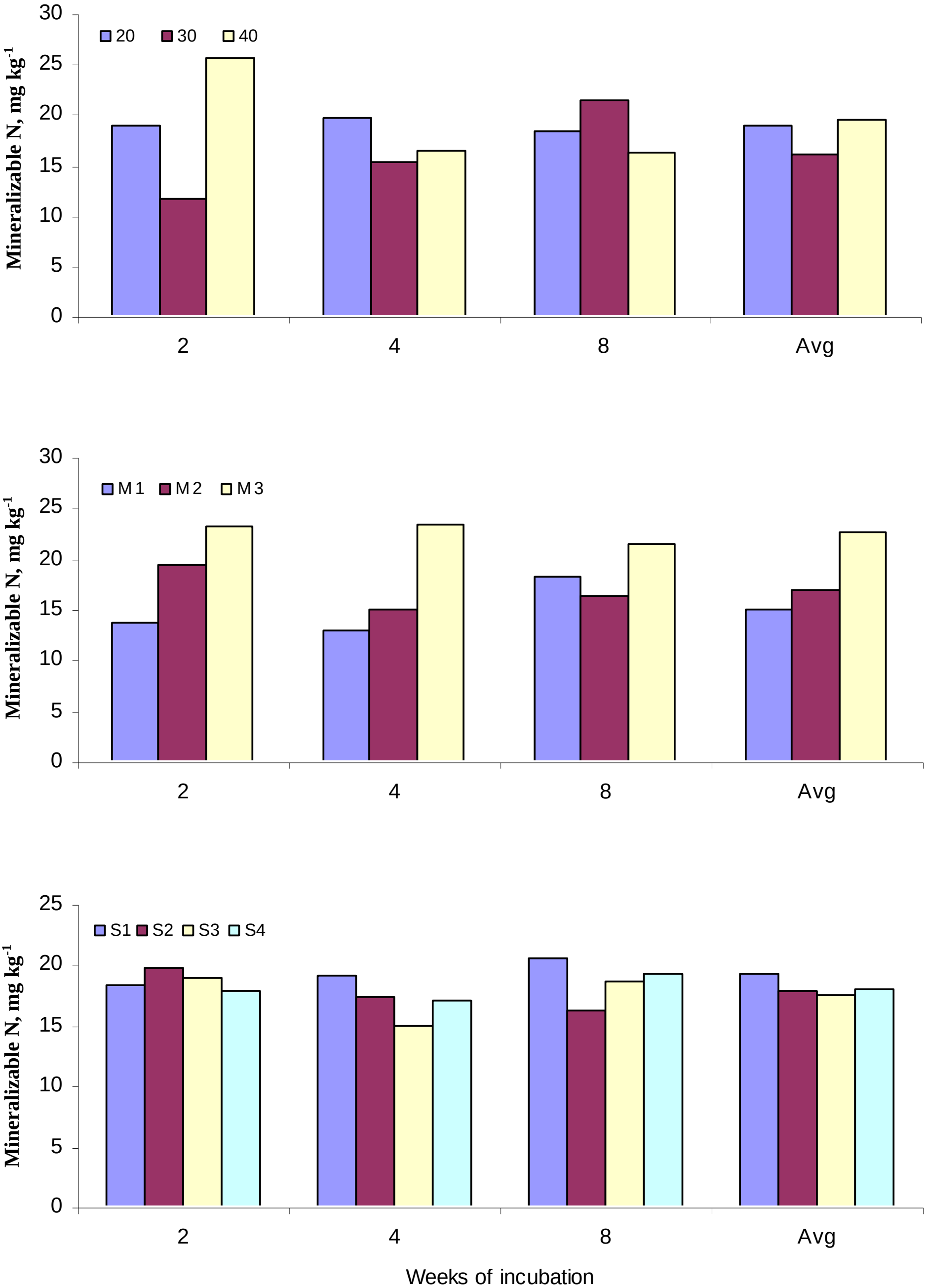

Fig. 4. Changes in mineralizable N of soils for 8 weeks at different temperature and moisture regimes. T_1-T_3, temperature regimes of 25, 30 and 40°C, respectively; M1-M3, moisture regimes of 15, 30 and 45%, respectively; S1-S4, salinity levels of 5, 7, 9 and 18 dSm^{-1}, respectively.

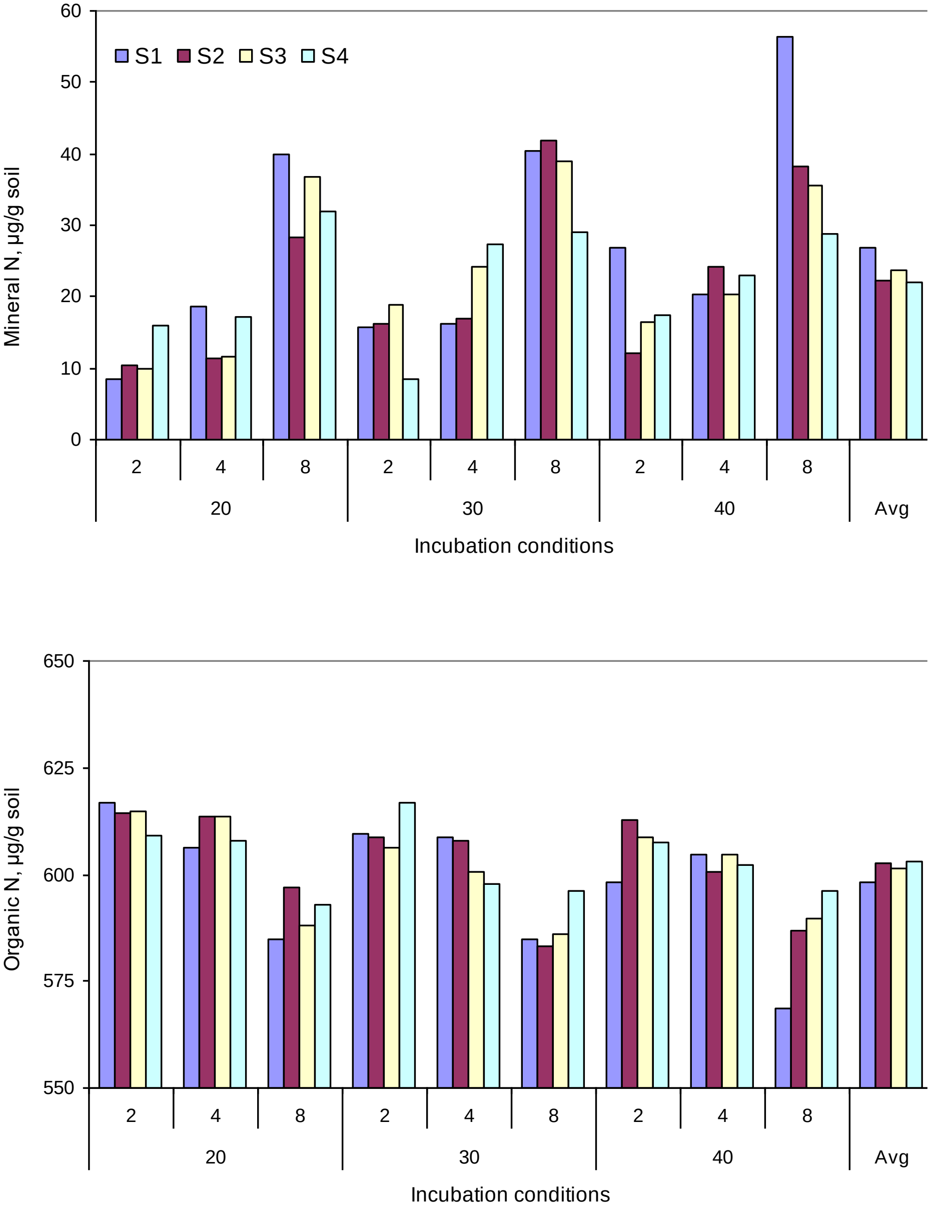

Fig. 5. Inorganic (mineral) and organic N content of soils incubated for 2, 4 and 8 weeks under different conditions of temperature (20, 30 and 40^{o}C) and salinity (S1, S2, S3 and S4; 5, 7, 9, and 18 dSm^{-1}, respectively).

Discussion

Supply of N limits the crop production more than any other nutrient element. Since a greater part of the plant N is derived from soil even in the presence of fertilizer N, mineralization and plant availability of N from native soil organic matter is a crucial factor in crop production. In addition, mineralization of N from organic matter applied to soil as green manures plays an important role in N nutrition of crop plants. Release/accumulation of mineral N in soil and its uptake by crop plants has been reported following incorporation of plant residues rich in total N i.e., legumes (Azam *et al*., 1993; Soon and Arshad, 2002).

Being a microbial process, mineralization of N is influenced by physical and chemical environment of soil. Of the different key factors, pH, amounts of different ions, temperature and moisture are reported to play a significant role in the mineralization and availability to plants of N from soil. In the present studies, mineral N increased with temperature, the increase being significantly higher at 30 and 40°C after 2 weeks of incubation. The effect diminished with time and after 8 weeks, amounts of mineral N were almost comparable at 30 and 40°C. The average values after 8 weeks were, however, higher for the two higher temperature regimes. Nevertheless, significant positive correlations were obtained between the amounts of N mineralized at different temperature regimes.

Although mineral N content of the soil increased with time at different moisture regimes, it was almost similar at 30 and 45% moisture when average values for factors other than moisture were computed, but significantly lower than that observed at a moisture content of 15%. Increasing soil salinity had a consistent negative effect on the accumulation of mineral N at all incubation intervals, although an increase with time was obvious. Retardation as well as complete inhibition of N mineralization in the presence of high salt concentrations has been reported (Malik & Azam, 1979; Sethi *et al*., 1993). Thus one of the effects of salinity on plant growth may be the reduced availability to plants of N from native and applied organic sources as a result of retarded mineralization. In the present studies, negative effect of salinity on N mineralization was mitigated to a significant extent by high temperature.

Nitrogen released from organic (native soil organic matter or that derived from plant residues) or inorganic (fertilizer N) sources not only provides the required amount of N, but exerts additional beneficial effects on processes that help plants grow better. For example, not only root proliferation is enhanced in the presence of ample supplies of N, but the root induced N mineralization adds to the availability of N (Azam *et al*., 1992a,b). This enhancement is brought about mainly through the “priming” effect of the added N (Jenkinson *et al*., 1985).

Not only the mineralization of N, but the nitrification of newly formed NH_4 is also inhibited under saline conditions as was the case in the present study. To a certain extent, negative effects of salinity on nitrification were mitigated at higher temperature regimes. Many studies have demonstrated inhibition of nitrification in the presence of salts (Westerman & Tucker, 1974; Sethi *et al*., 1993). Under conditions inhibitory to nitrification, some of the soil and plant processes may in fact be favoured due to the availability of NH_4 which is more beneficial to root proliferation, rhizodeposition and mineralization of native soil N. Under saline conditions that are inhibitory to nitrification as also observed in the present studies, the rhizodeposition is particularly enhanced to help mitigate the stress.

Under saline conditions, the process of nitrification is inhibited as a result of toxic ion effects on specific microbial functions. One of the factors responsible for decreased nitrification under saline conditions may be the reduced availability of CO_2 that results from respiratory activities of microorganisms. Negative effects of salinity on decomposition of organic matter (and thus the release of CO_2) have often been reported (Laura, 1974; Malik and Azam, 1979), while the process of nitrification is fairly sensitive to the availability of CO_2. The predominant nitrifiers i.e., Nitrosomonas, Nitrobacter and Nitrosospira etc., being autotrophs use CO_2 as the sole source of carbon (Aleem *et al*., 1985). Hence, availability of CO_2 can become a limiting factor for nitrification. At low CO_2 concentrations in soil, nitrification is significantly retarded, while it is reported to increase at elevated levels of CO_2 (Hungate *et al*., 1999).

In the present studies, the process of nitrification seemed to have been inhibited under high moisture conditions. It is customary to assume that this inhibition results from reduced availability of oxygen. It is logical to assume, however, that under these conditions, nitrification may be affected more by reduced supply of CO_2 than that of O_2 (Azam *et al*., 2004). This is particularly so as the nitrifiers meet most of their O_2 demands from water and hence level of molecular oxygen in the soil atmosphere may not be that important for nitrification to occur. Another factor that could be responsible for low amounts of NO_3 at higher moisture levels may be its loss through denitrification. Losses of N through denitrification under high moisture conditions (mainly because of reduced availability of O_2) are not uncommon (Bouwman, 1990). All the factors, might have contributed to almost complete inhibition of nitrification at 30 and 45% moisture in the present studies while significant quantities of NO_3-N accumulated at 15% moisture. In fact, under these conditions, nitrification inhibition is helpful in conserving N as any NO_3 formed will be susceptible to losses *via* leaching and denitrification.

The time period elapsed between incorporation of plant residues and introduction of plants may have significant influence on the availability of N originating from soil as well as from fertilizer and thus the growth performance of plants. Plant residues decomposing in soil play a key role in determining the net release and availability to plants of N from native organic matter and/or applied sources i.e., organic matter and fertilizer. In general, immobilization-mineralization turnover of N and the further fate of mineralized N (e.g., losses through denitrification) are regulated by the chemistry of the organic inputs, the process being slow with the increased complexity of the latter (Azam *et al*., 1985). Addition of organic matter (as *Sesbania* plant residues in the present studies) not only results in increased microbial activity leading to higher mineralization of organic N, but it may also cause greater loss of mineralized N through denitrification at the expense of easily oxidizable C (Azam *et al*., 2002; Pansu & Thuriès, 2003). In general, higher mineralization of N is recorded in soils amended with N-rich plant residues (Azam *et al*., 1993; Eriksen & Jensen, 2001; Seneviratne, 2000). Simultaneously, however, the mineralized N will be susceptible to losses in soil amended with plant residues containing significant amounts of easily oxidizable C and mineralizable N like *Sesbania* in the present study.

Potentially mineralizable N is a measure of plant available N. In the present studies, a higher content of mineralizable N was apparent at 20°C at the two lower levels of salinity. At the two higher salinity levels, however, more mineralizable N was determined in samples previously incubated at 30 and 40°C as compared to that at 20°C. The effect of moisture was variable, although relatively higher values of mineralizable N were obtained at 45% moisture in most cases. These observations suggest that under these conditions, the process of N mineralization was inhibited during the first phase of incubation and hence potentially mineralizable N was conserved. Upon the second incubation, this N got mineralized. Since, mineralization of N was inhibited under saline conditions, the second incubation of samples led to the determination of greater amounts of mineralizable N (Figs. 4 and 5). In contrast to the data presented in Figs. 1, 3 and 5, mineralizable N was not affected similarly at the three temperature regimes as the coefficients of correlation were either negative or non-significant when data sets for any two-temperature regimes were compared. This is because of the pre-incubation of soil samples for 0-8 weeks under different conditions and the resultant changes in mineralization of N.

A net and complete loss of NO_3-N (Fig. 5) is not unexpected especially from soil containing sufficient quantities of easily oxidizable organic matter (Azam *et al.*, 2002; Pansu & Thuriès, 2003). Under some of the incubation conditions (especially high moisture content during aerobic conditions), no losses of NO_3-N seemed to have occurred. In fact, these soil samples did not contain any NO_3 at the start of anaerobic conditions. Apparently, either the process of nitrification was inhibited or the NO_3-N formed was denitrified during the process of incubation under the influence of easily available C in the form of *Sesbania* plant material. Losses of NO_3-N under apparently aerobic conditions have often been reported as a result of microsite anaerobiosis (Bauhus *et al.*, 1996). The losses were more at higher temperature and significantly low at high moisture while the level of soil salinity did not show a consistent pattern of increase or decrease. As mentioned earlier, the so-called losses are in fact a reflection of the amount of NO_3-N present in soil samples before the start of anaerobic incubation. More NO_3-N accumulated at higher temperature and least at higher moisture.

Overall, the results obtained in the present study showed i) decrease in the mineralization of N under saline conditions and at higher moisture regimes, ii) increase in N mineralization at higher temperature, iii) loss of NO_3-N under high moisture conditions and iv) conservation of mineralizable N under high salinity and moisture conditions.

Acknowledgments

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