

# DIVISION S-3-SOIL MICROBIOLOGY AND BIOCHEMISTRY

Nitrogen Mineralization as Affected by Soil Moisture, Temperature, and Depth<sup>1</sup> K. G.

CASSMAN AND D. N. MUNNS<sup>2</sup>

## ABSTRACT

Variation in the rate of N mineralization in a Yolo soil profile was studied using *in vitro* incubation methods. Negligible amounts of  $\text{NH}_4\text{-N}$  were recovered from leachates, indicating that the rate of N mineralization was the primary factor controlling N availability. During a 13-week incubation at 25°C, 42% of the total estimated N mineralized was derived from the surface soil (0 - 18 cm), whereas 58% was contributed from the 18- to 108-cm depths. Cumulative N mineralization in the 0- to 18-cm and 18- to 36-cm depth samples followed first-order kinetics and was linear with respect to the square root of the incubation time. The mineralization rate constant differed more than twofold between the 0- to 18- and the 18- to 36-cm depth samples. At lower depths (36 - 72 and 72 - 108 cm) the relationship between cumulative N mineralization and the square root of time was curvilinear.

Interactive effects of soil temperature and moisture were also examined in an experiment with four incubation temperatures (15, 20, 25, and 30°C) in factorial combination with six soil moisture levels (0.1, 0.3, 0.7, 2, 4, and 10 bars). There was a significant moisture X temperature interaction; N mineralization increased above that expected from additive effects at 30°. Multiple regression was used to generate an equation that predicted net N mineralization as a function of soil temperature and moisture. The apparent effect of soil water content on N mineralization depended on experimental procedure. When soil water content was varied by adding water to air-dry soil without complete equilibration, N mineralization declined linearly with water content. In contrast, there was a sharp decline between 0.3 and 2-bar treatments and a more gradual decline at higher matric suctions when soil was equilibrated by pressure-membrane desorption before incubation. These results indicate that both quantity and distribution of soil water affect *in vitro* estimates of N mineralization.

*Additional Index Words:* soil nitrogen availability, soil water content, soil matric suction.

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EFFICIENT UTILIZATION of fertilizer N in crop production requires an accurate assessment of native soil N availability. Ideally N fertilization should supply N sufficient to make up the difference between available soil N and the N required for optimal yields. The amount of plant N derived from soil depends on the initial residual N present in the soil at planting and the mineralization of soil organic N during crop

growth. The former is easily measured as extractable  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  in soil at planting. The potential N mineralization is more difficult to estimate, due to variation in the soil profile and fluctuations of soil moisture and temperature.

The correlation between total soil N content and N mineralization is not good when comparing different soil types (Harmsen and Van Schreven, 1955). However, Stanford and Smith (1972) studied N mineralization rates in 39 diverse soils and reported: (i) a consistent correlation between the N mineralization rate and the quantity of mineralizable N estimated from a first order rate equation, (ii) a linear relationship between cumulative N mineralization and the square root of the incubation time and, (iii) a similar mineralization rate constant in most soils. In a subsequent study N mineralization rates at depths to 45 cm in eight Idaho soils were also found to be linearly related to the square root of the incubation time, although it is not clear that the first-order rate constants were similar at the different soil depths (Stanford et al., 1974). This work demonstrates the feasibility of assessing N mineralization from incubation studies. Our motive was to see if assessment could be improved by eliminating some of the potential sources of error.

We know of only one report concerning interactive effects of soil moisture and temperature on the rate of native soil N mineralization. In this study, neither soil moisture (ranging from 0.3 to 10 bars) nor temperature (10 or 22°C) were found to affect N mineralization during a 2-week incubation (Justice and Smith, 1962). This finding conflicts with work on the separate effects of soil moisture or temperature. In general mineralization rates are reported to be proportional to soil water content in the range of 0.2 to 15-bar matric suction and maximal at approximately field capacity (Greaves and Carter, 1920; Robinson, 1957; Reichman et al., 1966; Miller and Johnson, 1964; Stanford and Epstein, 1974). However, in all of the above studies, different soil moisture content was accomplished by mixing water with air-dry soil, followed by incubation. This wetting procedure is unlikely to produce uniform water distribution, especially at low soil water contents. When soil temperature effects were considered at optimal soil moisture levels, Stanford et al. (1973) found similar N mineralization rate constants in 11 different soils for each temperature studied in the 5 to 35°C range and an overall  $Q_{10}$  of approximately 2.

Our objectives were to examine the interactive effects of soil moisture and temperature, to test the effect of soil water uniformly distributed by pressure-membrane equilibration, and to compare the kinetics of N mineralization at different depths in the soil profile.

<sup>1</sup>Contribution from the Dep. of Land Air & Water, Univ. of California-Davis, CA 95616. Received 24 Apr. 1980. Approved 17 June 1980.

<sup>2</sup> Postgraduate Research Scientist and Professor of Soil Science.

## MATERIALS AND METHODS

The soil, Yolo series (silty-loam, mixed, nonacid, thermic Typic Xerorthent), was from the agronomy research plots at the University of California at Davis. The previous summer the field had been planted with sudangrass (*Sorghum bicolor* L. 'Moench'), which was cut and removed in the fall. Soil samples were collected 30 May 1979 from an unfertilized soybean (*Glycine max* L. 'Merr.') plot 2 weeks after emergence. At each of three sites within the field a soil core was taken with an 8-cm diameter auger and separated into four samples representing depths of 0 to 18, 18 to 36, 36 to 72, and 72 to 108 cm. Samples were air-dried and screened through a 2-mm sieve. A water retention curve was determined for each sample by pressure-membrane desorption (Richards, 1954). Total N content was determined by Kjeldahl digestion and colorimetric analysis of the distillate for  $\text{NH}_4\text{-N}$  (Mitchell, 1972). Initial extractable  $\text{NH}_4\text{-N}$  was also measured colorimetrically in distillates of 1.0M KCl soil extracts (25 g of soil: 50-ml extract, 1-hour shaking time). Initial extractable  $\text{NO}_3\text{-N}$  was measured in the same 1.0M KCl extracts by the cadmium reduction method (APHA, 1975). Soil pH was measured with a glass electrode from a 1:2 soil/ water suspension.

### Mineralization at Different Depths

The three soil samples collected from each depth were prepared separately in leaching tubes using the method of Stanford and Smith (1972) with some modifications. Samples were mixed with 40-mesh silica sand 1:2 (wt/wt soil:sand oven-dry mass basis). Water contents corresponding to matric suctions of 0.1 and 0.3 bar were determined for each soil-sand mixture as previously described. In each leaching tube a small piece of glass wool was placed over the bottom stem and a 10-g layer of 40-mesh silica sand poured on top. Then 90 g of the soil-sand mixture was poured in and another thin piece of glass wool placed on top. Dilute nutrient solution (1.0 mM  $\text{CaCl}_2$ , 1.0 mM  $\text{MgSO}_4$ , and 1.0 mM  $\text{KH}_2\text{PO}_4$ ) was added to the soil-sand mixture in each tube such that the initial soil water content corresponded to 0.24-bar matric suction. Tubes were not leached initially. Their tops were covered with aluminum foil. Gas exchange with the atmosphere was facilitated by making two pinholes in the aluminum foil and leaving the bottom stems open. Tubes were incubated at  $25 \pm 0.5^\circ\text{C}$ . Mineralized N was recovered from each tube after 1, 2, 3, 4, 5, 7, 9, and 13 weeks by leaching with 100 ml of dilute nutrient solution in 50-ml increments. Leachates were analyzed directly for  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  as previously described. After each leaching the water content of each soil-sand sample was restored to its original level by suction. Loss of water was negligible between leachings.

### Effects of Temperature and Moisture

Three experiments were done with a composite surface soil sample prepared by mixing equal amounts of the 0- to 18-cm soil samples.

A preliminary experiment was done to ascertain that imposition of high pressures in the pressure membrane apparatus had no adverse effects on subsequent mineralization. Samples of 25-g air-dry soil were pretreated by equilibration at 10, 4, 2, 0.7, and 0.3 bar and then brought to either 2 bars or 0.3 bar for analysis and incubation. For each treatment sequence, six soil samples were run: two were used to determine the initial soil water content, two were analyzed immediately for initial soil  $\text{NO}_3\text{-N}$  before incubation, and two were incubated at  $25 \pm 0.5^\circ\text{C}$  for 2 weeks in 125-ml Erlenmeyer flasks covered with parafilm that was pierced with two pinholes. Water loss was negligible during incubation. Initial and final soil  $\text{NO}_3\text{-N}$  was measured in 1.0M KCl extracts as described previously. Net N mineralization was calculated by subtracting the initial soil  $\text{NO}_3\text{-N}$  present before incubation from the final soil  $\text{NO}_3\text{-N}$  recovered after incubation.

In a second experiment, interactive effects of soil temperature and moisture were examined with four incubation temperatures (15, 20, 25, and  $30^\circ\text{C}$ , all  $\pm 0.5^\circ\text{C}$ ) in factorial combination with six soil moisture levels. Approximately 25 g of air-dry soil was equilibrated once at 10, 4, 2, 0.7, 0.3, or 0.1 bar by the pressure-membrane method. For each soil moisture level, 12 soil samples were run: two were used to determine the initial soil water content, two were analyzed immediately for initial soil nitrate content, and replicate samples were incu-

bated for 2 weeks at each of the four temperatures in Erlenmeyer flasks as described previously. Initial and final soil  $\text{NO}_3\text{-N}$  were measured as before, and net N mineralization was calculated as described above.

A third experiment compared the net N mineralization in soil samples brought to different average water contents by addition of distilled water to air-dry soil. Water in quantities corresponding to 10-, 4-, 2-, 0.7-, 0.3-, and 0.1-bar suction was added drop by drop to 25 g of air-dry soil and thoroughly mixed with a spatula. Duplicate samples were incubated at  $25 \pm 0.5^\circ\text{C}$  for 2 weeks as described earlier. Initial soil  $\text{NO}_3\text{-N}$  was determined from duplicate 25 g of air-dry samples. Final soil  $\text{NO}_3\text{-N}$  after incubation and net N mineralization were calculated as before.

## RESULTS AND DISCUSSION

Water retention curves varied little with depth, except at 72 to 108 cm, where the soil graded into a fine sandy-loam resulting in lower water retention (Fig. 1). With increasing depth, total N content and extractable  $\text{NO}_3\text{-N}$  decreased, extractable  $\text{NH}_4\text{-N}$  was uniform, and soil pH increased slightly (Table 1).

### Mineralization at Different Depths

Recovered  $\text{NH}_4\text{-N}$  in leachates was less than  $0.5 \mu\text{g NH}_4\text{-N/g soil}$  throughout the entire incubation period in all samples, consistent with the supposition that  $\text{NH}_4\text{-N}$  levels in aerated soils are generally negligible during crop growth (Harmsen and Van. Schreven, 1955). Therefore, our emphasis is on the rate of  $\text{NO}_3\text{-N}$  release.

Cumulative N mineralization in the 0- to 18- and 18- to 36-cm samples was linear with respect to the square root of the incubation time (Fig. 2). These results agree with others (Stanford and Smith, 1972; Stanford et al., 1974). At lower depths the relationship between cumulative N mineralization and the square root of the incubation time was curvilinear. Stanford et al. (1974) reported that the correlation coefficients for linear regression of cumulative N mineralization on the square root of the incubation time generally decreased as the sampling depth increased. These results suggest curvilinearity associated with depth or low mineralizable N.

Evaluating the data using a first-order rate equation and the iterative process described by Stanford and Smith (1972) gives values of  $121 \mu\text{g N/g soil}$  potentially mineralizable for 0 to 18 cm and  $30 \mu\text{g/g}$  for 18 to 36 cm. The corresponding mineralization rate constants were  $0.04 \pm 0.001$  and  $0.09 \pm 0.001 \text{ week}^{-1}$  ( $P_{0.05}$ ). This substantial difference in the mineralization rate constant at different depths suggests that it is not always appropriate to use one universal rate constant when estimating soil N availability from in vitro N mineralization studies, as has been proposed (Stanford et al., 1974; Stanford et al., 1977).

The estimated relative contribution to the total N mineralized in the 0- to 108-cm profile would be respectively 42, 18, 25, and 15% for the 0- to 18-, 18- to 36-, 36-, to 72-, and 72- to 108-cm depths, assuming uniform bulk density throughout the soil profile. Although the largest fraction would be derived from the surface soil (0-18 cm), nearly 60% would be derived from 18- to 108-cm depths. This indicates that evaluations of soil N availability in agricultural soils should include the potential contribution from the subsoil. Actual crop uptake of mineralized N would

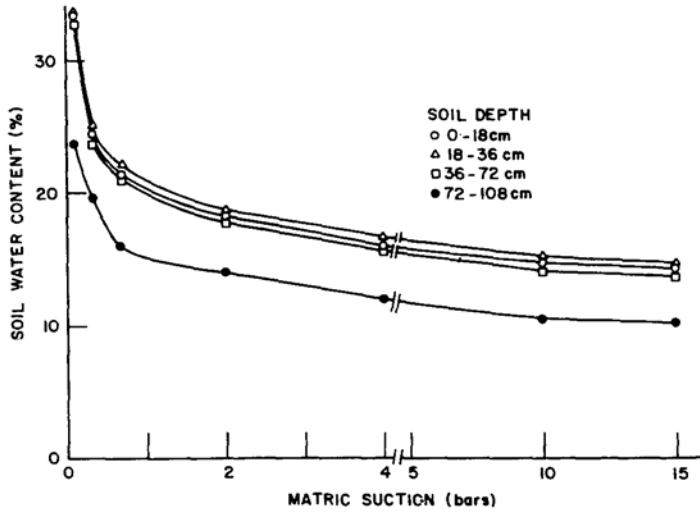


Fig. 1—Water retention curves at four depths in the Yolo soil profile.

be more difficult to estimate, because uptake efficiency may vary with soil depth.

**Effects of Temperature and Moisture**

The pressure-membrane equilibration method was employed to ensure that soil samples were equilibrated with respect to soil water distribution before incubation began. Pressure-membrane equilibration pretreatments did not significantly affect subsequent net N mineralization after re-equilibration at 0.3 bar (Table 2). The slight reduction in soil pre-equilibrated at 10 and 4 bars may be attributable to increased removal of water-soluble N compounds with water removal during pressure-membrane equilibration (Bremner, 1965).

Both soil moisture and temperature treatments were chosen to encompass levels normally encountered in irrigated fields. There was a significant interactive effect of soil moisture and temperature on net N mineralization (Fig. 3a and 3b). At suboptimal soil moisture levels there was increased net N mineralization in the 30°C treatment above that expected from strictly additive effects. The existence of a soil temperature x moisture interaction indicates that these factors should not be considered independently.

Mineralization was maximal in the 0.3-bar treatment and decreased in the 0.1-bar treatment at all four temperatures (Fig. 3a). Similar results have been reported by others (Miller and Johnson, 1964; Reichman et al., 1966; Stanford and Epstein, 1974). There was a sharp decrease in net N mineralization between the 0.3- to 2-bar treatments and then a gradual decline over the 2- to 10-bar range at all temperatures. The decline in the net N mineralization rate was not pro-

**Table 1—Initial soil N status and pH of the Yolo silt-loam at different soil depths.**

Depth cm	Total N %	Extractable		pH
		NH <sub>4</sub> -N μg/g	NO <sub>3</sub> -N μg/g	
0-18	0.12	1.2	7.1	7.2
18-36	0.09	0.9	4.3	7.6
36-72	0.06	1.1	3.2	7.6
72-108	0.04	1.1	1.7	7.7

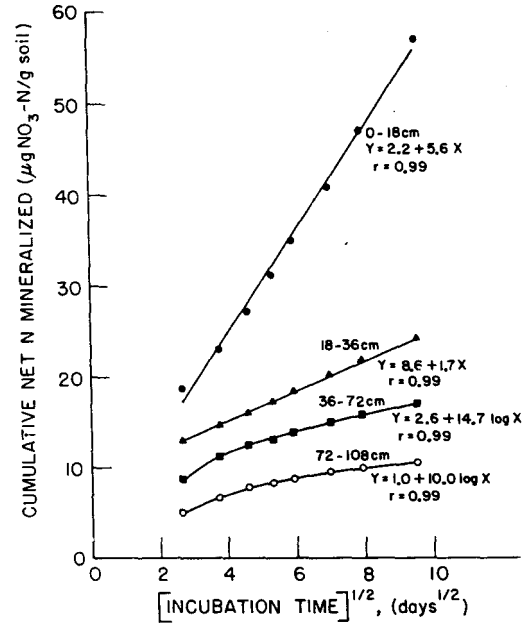


Fig. 2—Cumulative N mineralization in soil samples from different depths in the Yolo soil profile during a 13-week incubation at 25°C.

portional to the decrease in soil water content over the 0.3- to 10-bar range (Fig. 3b). This result conflicts with those of Greaves and Carter, (1920), Robinson, (1957), Miller and Johnson, (1964), Reichman et al., (1966), and Stanford and Epstein, (1974), all of whom mechanically mixed water with air dry soil to achieve different soil moisture levels. The distribution of water in soil samples prepared by this "water addition method" may not be uniform, especially at low soil water contents. At low soil water contents the actual distribution of soil water may affect the rate of N mineralization. Lees and Quastel (1946) showed that the nitrification rate was proportional to the soil surface area on which the NH<sub>4</sub>-N is adsorbed.

To test whether the method of adjusting water content affects the mineralization rate, the incubation experiment was repeated using the water addition method. At soil water contents corresponding to matric suctions greater than 0.7 bar, soil water distribution was visibly heterogeneous even after exhaustive mixing. After 2 weeks' incubation at 25°C, there was a proportional decline in net N mineralization as soil water content decreased from values corresponding to

**Table 2—Effect of pressure-membrane equilibration on net N mineralization † in the Yolo silt-loam.**

Pretreatment matric suction	Final matric suction	Net N mineralized
10	0.33	9.6
4	0.33	9.5
2	0.33	11.4
0.7	0.33	11.9
0.33	0.33	10.7
2	2	5.8
0.33	2	5.2
		L.S.D. 0.05
		2.9

† Soil samples were equilibrated twice in a pressure-membrane apparatus, first in a pretreatment equilibration followed by a final equilibration, then incubated at 25°C for 2 weeks.

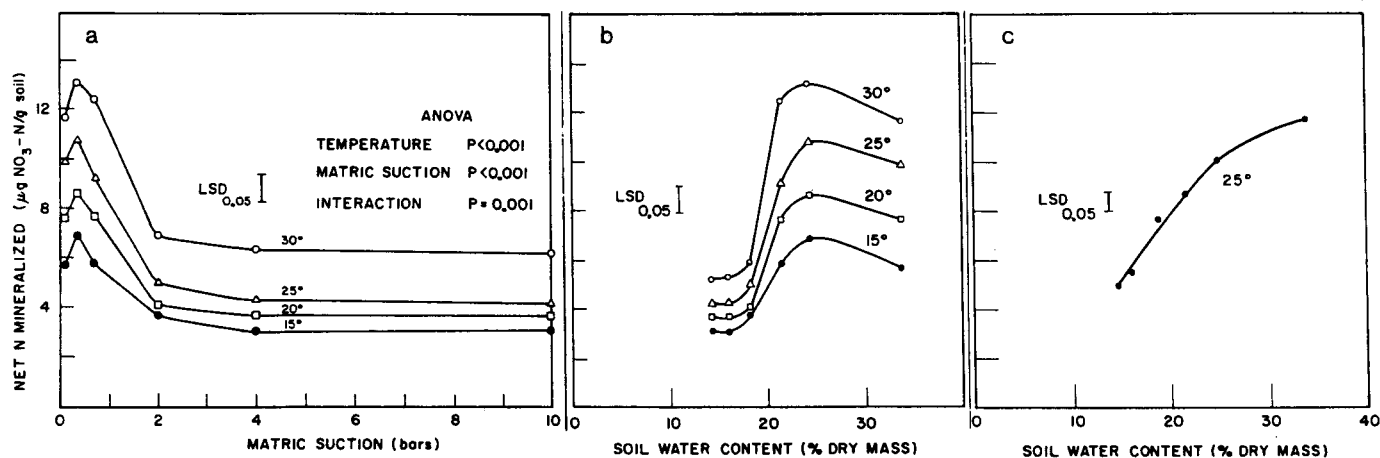


Fig. 3—Effect of soil moisture and temperature on net N mineralization in Yolo surface soil. Data in *a* and *b* were obtained from an experiment in which soil moisture levels were established by the pressure-membrane method. Data in *c* were obtained from another experiment when soil moisture levels were established by the water addition method.

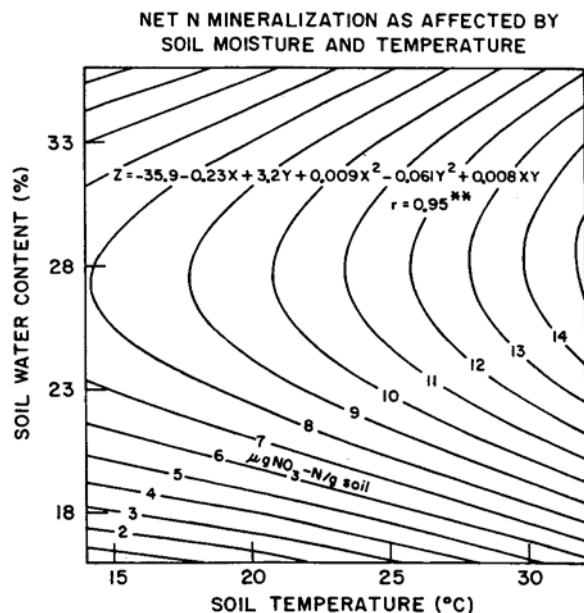


Fig. 4—Multiple regression of net N mineralization in the Yolo surface soil as a function of soil temperature and water content. (Data from Fig. 3b).

0.3- to 10-bar matric suction (Fig. 3c) as reported by the other investigators cited above.

Between irrigation cycles, fluctuations in soil moisture content in the field occur by desorption. The pressure-membrane equilibration method should give results that would better approximate actual field conditions, provided pressurized pretreatment does not adversely affect the activity of the microorganisms studied. More uniform water distribution is obtained at low soil water contents, and less puddling occurs at higher water contents.

Soil samples for this study were collected from an irrigated field (planted in soybeans) 2 weeks after emergence. During crop growth, soil water content was monitored at all four depths sampled in the 0- to 108-cm profile. Weekly average soil water contents ranged from values that correspond to a range of 1.7 to 0.4-bar matric suction (unpub. data, K. G. Gassman). Thus, the critical soil moisture range for assess-

ing N mineralization in the field was between 2 and 0.1 bar. With the use of the data from Fig. 3a and 3b, multiple regression was employed to generate an equation that could predict net N mineralization as a function of soil temperature (between 15 and 30°C) and water content (between 18 and 34%). The net N mineralization occurring at any soil temperature-moisture combination in those ranges was predicted by:

$$N = -35.9 - 0.23x - 3.2y - 0.009x^2 - 0.061y^2 + 0.008xy \quad (r = 0.95)$$

where *N* represents the net N mineralized during a 2week incubation, *x* denotes soil temperature, and *y* denotes soil water content (Fig. 4). The validity of this equation for use in estimating soil N mineralization in the field has not yet been established.

## CONCLUSIONS

In a Yolo soil, the kinetics of N mineralization is affected by (i) the depth in the soil profile, (ii) a temperature  $\times$  moisture interaction, and (iii) both the quantity and distribution of soil moisture. The Yolo soil may not be atypical in these respects. The ability to assess soil N availability might be improved if these effects were evaluated for other soil types.

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