MINERALIZATION OF NITROGEN IN TWO
SOIL-ORGANIC-MATTER FRACTIONS
EXTRACTED WITH FORMIC ACID

by HASSAN SULIMAN IBRAHIM

Guneid Research Substation, Agricultural Research Corporation,
Guneid, Republic of the Sudan

SUMMARY

Extraction of a calcareous meadow soil with boiling formic acid containing 10 per cent acetylacetone yielded a fraction of soil organic matter representing about 40 per cent of the total. This fraction contained only 2.2 per cent N. Less than 18 per cent of this was mineralized during incubation for 8 weeks at 28°C. Further extraction of the soil with a formic acid and hydrofluoric acid mixture yielded a second fraction. This represented about 37 per cent of the total organic matter and contained 6.0 per cent N of which up to 41 per cent was mineralized during incubation. Preincubation and leaching had a stabilizing effect on subsequent mineralization of native N of a standard sandy loam soil.

INTRODUCTION

The studies of Tinsley and co-workers 4 5 8 9 showed that about half the organic matter can be extracted from soils by boiling with anhydrous formic acid (98%) and that the amount of organic matter extracted can be increased by adding various other reagents. Parsons and Tinsley 4 5 added lithium bromide and Tinsley and Walker 8 used fluorboric acid. Later Adatia 1 used acetylacetone and more recent, unpublished work by Tinsley at Aberdeen University showed that after a first extraction of a calcareous meadow clay loam soil with formic acid containing 10 percent acetylacetone, a second extraction with formic acid containing hydrofluoric acid released an even larger proportion of the total organic matter.

In the work described here quantities of these two fractions were isolated and used to study the rate of mineralization of nitrogen in
each when mixed with a standard sandy loam soil of relatively low organic matter content which was then incubated for up to 8 weeks.

MATERIALS AND EXPERIMENTAL PROCEDURE

Soils
The standard sandy loam (described in Table 1) was prepared for the first experiment by air-drying and screening through a 2-mm sieve, whereas for the second experiment this soil was preincubated and leached with water to reduce the initial level of mineral nitrogen present. The soil was analysed by routine methods; pH was measured in a soil suspension (1: 2.5 soil/water); mechanical analysis by the international pipette method described by Piper 6; organic carbon by the wet oxidation method of Tinsley 7; total nitrogen by the micro-Kjeldahl method and the maximum waterholding capacity by a method essentially similar to that used by Jansson 3.

Extraction of the organic matter fractions with formic acid
The soil from which the organic matter fractions were extracted was a calcareous meadow soil from Reading University Farm. It is described in Table 1. The soil was air dried and screened through a 2-mm sieve and prior to extraction of organic matter, plant residues in the soil were removed by flotation on carbon tetrachloride several times. The root-free soil was allowed to dry at room temperature until free from carbon tetrachloride. Now replicate 20-g samples of this 'root free' soil were taken treated with 135 ml of 98% formic acid and 15 ml of acetylacetone, then boiled under a reflux cold finger condenser for 15 minutes. After cooling and centrifuging, the supernant extract was mixed with 5 times its volume of di-isopropyl ether containing 1% acetyl chloride and left to stand for 1 hour after which the whole material was centrifuged and the supernatant liquid was decanted. This process of dissolution and precipitation of the organic matter was repeated, using decreasing amounts of formic acid: 100, 80, 60, and 30 ml. In the sixth and final precipitation only di-isopropyl ether was used without acetyl chloride. The organic matter obtained was washed twice with diethyl ether and once

<table>
<thead>
<tr>
<th>TABEL 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description of the sandy loam and meadow soils</td>
</tr>
<tr>
<td>Mechanical analysis ( % mineral matter)</td>
</tr>
<tr>
<td>Soil</td>
</tr>
<tr>
<td>Sandy loam soil</td>
</tr>
<tr>
<td>Meadow soil</td>
</tr>
</tbody>
</table>
N-MINERALIZATION IN SOIL-ORGANIC-MATTER FRACTIONS

TABLE 2

Composition of the organic-matter fractions

<table>
<thead>
<tr>
<th>Organic fraction</th>
<th>Ash-free yield %</th>
<th>Ash %</th>
<th>N %</th>
<th>Ash-free C %</th>
<th>C %</th>
<th>C:N</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First experiment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First fraction</td>
<td>41.0</td>
<td>10.94</td>
<td>2.25</td>
<td>2.50</td>
<td>51.36</td>
<td>22.8</td>
</tr>
<tr>
<td>Second fraction</td>
<td>36.0</td>
<td>4.25</td>
<td>6.05</td>
<td>6.93</td>
<td>52.45</td>
<td>7.9</td>
</tr>
<tr>
<td><strong>Second experiment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First fraction</td>
<td>38.1</td>
<td>8.42</td>
<td>2.23</td>
<td>2.42</td>
<td>53.36</td>
<td>23.8</td>
</tr>
<tr>
<td>Second fraction</td>
<td>37.6</td>
<td>7.17</td>
<td>5.965</td>
<td>6.39</td>
<td>52.70</td>
<td>8.8</td>
</tr>
</tbody>
</table>

with petroleum spirit (40–60 B.P.), then dried in a vacuum dessicator, ground in an agate-mortar machine, and stored in a tightly closed sample tube.

The second fraction was obtained by treating the soil residue (remaining after the first extraction with formic acid) with 100 ml formic acid and 25 ml 40% hydrofluoric acid and refluxed in a boiling water bath for 30 minutes. The procedure of dissolution and precipitation of the organic matter mentioned above, was repeated several times using formic acid, di-isopropyl ether, and acetyl chloride. In the first precipitation only, acetic anhydride was used in addition to the chemicals described above.

Analysis of the organic fractions

Total nitrogen and organic carbon were determined by the methods already cited on samples of 40 mg and 20 mg respectively. Ash was determined by ignition to constant weight at 450°C in an electric muffle furnace. Results are given in Table 2.

Dissolution and neutralization of the organic fractions

To ensure that the organic fractions extracted from the clay soil could be distributed evenly through the sandy loam soil in a manner easily accessible to micro-organisms, it was necessary first to dissolve each in water by neutralizing with sufficient N KOH.

Standard solutions of both first and second organic fractions were prepared for each incubation experiment as follows: 22.222 and 7.519 g of the first and second organic fractions respectively were weighed in separate 400 ml beakers for the first experiment. Then 100 ml distilled water were added to each beaker, mixed well with a stirrer and left to soak for 2 hours. The material was stirred and its pH was read, then adjusted to pH 6.5 by addition of normal KOH from a microburette. The whole material was transferred to 250-ml volumetric flask and made up to volume. It was found that the first organic fraction did not dissolve completely in the 250 ml in the first experiment and so more water was added. The second fraction dissolved readily in the 250 ml
volume. In the second experiment, the weights of organic fractions taken for the preparations of standards were 22.300 and 8.389 of the first and second fractions respectively.

The nitrogen contents of each solution were determined in 5-ml samples by the Kjeldahl method using 5 ml concentrated H₂SO₄ and 1 g catalyst mixture and distilling the whole digest. The results of the nitrogen determinations are:

<table>
<thead>
<tr>
<th></th>
<th>First fraction</th>
<th>Second fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml KOH</td>
<td>mg N/ml</td>
</tr>
<tr>
<td>First experiment</td>
<td>88.4</td>
<td>1.54</td>
</tr>
<tr>
<td>Second experiment</td>
<td>104.0</td>
<td>1.87</td>
</tr>
</tbody>
</table>

**Technique and conditions of incubation**

In the two experiments carried out the rates of nitrogen applications per 50 g air dried soil were:

- N₀ = control; N₁ = 8 mg N; N₂ = 16 mg N and N₃ = 24 mg N.

The amounts of organic fractions and water required to provide 50% waterholding capacity were measured in a syringe graduated from 0–20 ml. For the second experiment the organic fractions were ultrasonically mixed to produce a very fine suspension of the organic material. The contents of the syringe were injected into a polythene bag, similar to that described by Enno², containing 50 g of the standard sandy loam soil prepared as above. Great care was taken to distribute the material throughout the soil sample as evenly as possible. The organic matter was then mixed well with the soil and the bags were sealed with rubber bands. Corresponding samples treated with urea were prepared in a similar way. The rates of nitrogen applied in case of urea treated samples were:

- N₀ = 0 mg N; N₁ = 8 mg N; N₂ = 16 mg N; and N₃ = 24 mg N. Eight replicate soil bags for each treatment were transferred to an incubator (set at 28°C) for the required periods. Duplicate samples of each treatment were withdrawn from the incubator after one, two, four, and eight weeks for mineral-N determinations. Mineral N was extracted with 500 ml of 2M KCl solution. NH₄–N was determined by using 10% MgO suspension and NO₃–N by treating the same sample subsequently with a mixture of 15% titanous sulphate and ferrous sulphate. The results are shown in Figs. 1–8.

**DISCUSSION OF RESULTS AND CONCLUSIONS**

The first experiment showed that the sandy loam soil of the control (i.e. no added nitrogen) had a high nitrifying capacity even after eight weeks of incubation (Fig. 1). In the second experiment, in which the soil was preincubated, less NO₃–N was produced in the first two weeks of incubation as Fig. 5 shows. Surprisingly, after four weeks of incubation the preincubated soil had accumulated about the same amount of NO₃–N as in the non-preincubated soil.
Key to Figs. 1–8

**KEY**

- **NH₄⁻N (Urea)**
- **NO₃⁻N**
- **NH₄⁻N** (First)
- **NO₃⁻N** (Fraction)
- **NH₄⁻N** (Second)
- **NO₃⁻N** (Fraction)

![Graph](image)

**Fig. 1.** First experiment. Ammonium and nitrate N. Control soil (N₀).

![Graph](image)

**Fig. 2.** First experiment. Ammonium and nitrate N. N₁ level.
However, after eight weeks of incubation there was no further significant change in preincubated soil whereas in non preincubated soil there was further accumulation of NO₃–N between 4 and 8 weeks. It seems that preincubation followed by leaching with water had a stabilizing effect on subsequent nitrification in the N₀ control soil.

Both the first and the second experiment showed that soil samples treated with urea contained the largest amount of mineral-N after one week of incubation (Figs. 1–8).

All levels (i.e. N₁, N₂ and N₃) of both the first and second organic-matter fractions yielded their largest amounts of NO₃–N after eight weeks of incubation (Figs. 2, 3, 4, 6, 7, and 8). In the second experiment there was a steady increase of NO₃–N in all the different treatments of both the first and the second fractions. But after the fourth week slight loss of NO₃–N was observed from the second fraction, as in the control soil (N₀).

The first fraction showed only very small accumulation of NO₃–N between the fourth and eight week of incubation.

The total percentage of nitrogen mineralized from the first and second fractions did not show big differences between the different
Fig. 4. First experiment. Ammonium and nitrate N. N₃ level.

Fig. 5. Second experiment. Ammonium and nitrate N. Control soil (N₀).
Fig. 6. Second experiment. Ammonium and nitrate N. N$_1$ level.

Fig. 7. Second experiment. Ammonium and nitrate N. N$_2$ level.
levels of application after eight weeks of incubation in either the first or the second experiment.

In both experiments, more mineral-N was formed from the second fraction than from the first. This corresponds with lower C/N ratio (C/N 8.35) of the second fraction and indicates that the second fraction is more susceptible to microbial attack than the first. The maximum percentage of total mineral-N released from the first organic matter fraction, under the conditions of incubation used, ranged from 14 to 18 per cent according to treatment. Thus 82 per cent or more of the organic-N of this fraction remained 'resistant' to mineralization. Conversely, from 34 to 41 per cent of the total N was mineralized from the second fraction indicating that it was more susceptible to microbiological attack under the conditions used.
ACKNOWLEDGEMENTS

The author is grateful to Dr. J. Tinsley Head Department of Soil Science, University of Aberdeen, for his constant help and advice during the execution of this work. He is also indebted to Dr. F. Cope, Technical Assistance Expert, International Atomic Energy Agency of the United Nations and adviser to the Government of the Sudan for scrutinizing the manuscript.

Received July 21, 1970. Revised August 1971

REFERENCES