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144. EFFECT OF LIMING OF AN ACID, PEAT SOIL ON MICROBIAL ACTIVITY

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In order to investigate the effect of liming of an acid peat soil on plant growth, availability of soil nitrogen, and soil microflora, an experimental field was laid out in the spring of 1941 on a lowmoor peat containing about 60 percent organic matter and 40 percent clay. The pH of this soil was about 4.2. Calcium carbonate was applied at the rate of 2, 5, 10, 30 and 60 tons per ha. In order to determine the amount of nitrogen liberated as a result of liming, fertilizer nitrogen was applied in different pH levels an approximate estimate was made of the amount of soil nitrogen liberated by increased microbial activity after raising the pH. In addition nitrogen analyses of the crop were performed in some cases. The experiments were continued until 1944, when owing to war activities the polder in which the experimental field had been laid out, was flooded by the Germans. In 1946 the experiment was recontinued.

pH of the soil and organic matter content.

Table I shows the effect of liming on pH of the soil and organic matter content.

| Tons of CaCO | F | H of soil | percent organic matter | | |
|-------------------------------|--|-----------------------------------|---|--|--|
| per ha (applied in 1941). | 1941 (autumn) | 1944 | 1947 | 1944 | 1947 |
| 0 2 5 10 30 60 | 4.1 4.4 5.3 LANE 5.0 6.0 * DB | 4.3 4.5 6.6 6.9 10THL | 4.4 4.6 4.6 4.8 6 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 | 57.9 59.0 57.7 57.1 55.5 54.2 | 58.3 59.0 58.1 58.4 57.5 56.2 |

TABLE I. Effect of liming of an acid peat soil on pH and organic matter content.

It will be seen that large applications of lime have been required to raise the pH of this peat soil to values above six. In course of time the effect of the lower amount of lime disappears.

Although no organic matter determinations were carried out before 1944, it may be concluded from the available figures, that the plots with the highest rate of $CaCO_3$ have lost about 2—3 percent of their organic matter within three years after liming. After that period of time no further decrease of organic matter content on the limed plots has been observed, which indicates that apparently only a small part of the organic matter in this acid peat soil is decomposable after liming.





Fig. 1. Effect of liming in 1941 on response to fertilizer nitrogen (potatoes 1943); 1 q = 100 kg.

Fig. 2. Effect of limiting in 1941 on response to fertilizer nitrogen (rye 1944); I q = 100 kg.



Fig. 3. Uptake of nitrogen by potatoes (kg N per ha in tubers) on soils supplied with various amounts of CaCO₈ in 1941 and various amounts of fertilizer nitrogen in 1943.

Effect of liming on nitrogen availability.

The effect of liming on availability of soil nitrogen was studied by determining the responses of potatoes and cereals to fertilizer nitrogen at different lime levels. In order to prevent differences in availability of magnesium, phosphate and potassium due to liming, ample dressings of these nutrients were applied.

In the first year after liming slight indications were obtained that neutralization of the acid peat soil gave an improved nitrogen uptake by the plants. In the three following years this increased uptake of soil nitrogen was much more pronounced, as may be seen from Fig. I and 2. The potatoes of 1943 were analyzed for total nitrogen. These nitrogen values show that owing to liming four years ago more than 40 kg of soil nitrogen were absorbed by the potatoes (Fig. 3). Since only part of the liberated nitrogen will have been absorbed by the plants, it may be assumed that the real amounts of soil nitrogen liberated during one year have been more than twice as high.

When the experiment was recontinued in 1946 the effect of liming on the nitrogen nutrition was still evident. In the years 1947, 1948 and 1949 this effect had almost disappeared. These results are in accordance with the organic matter determinations (Table 1). Whether this drop in rate of decomposition of the organic matter is only of a temporary character or is due to the high grade of resistance against microbiological decomposition of the greater part of the organic matter is unknown.

Although the increased uptake of soil nitrogen by the plants in the first four years after neutralization may be partly ascribed to an improved absorption capacity of the roots, no doubt the main effect was due to a much enhanced liberation of nitrogen due to the activity of soil microorganisms. This may be concluded from the fact that on the acid soil normal plant growth was obtained when fertilizer nitrogen had been applied only and further from the fact that in the years 1947, 1948 and 1949 practically no effect of neutralization on nitrogen uptake has been observed.

Effect of liming on the microflora of the soil.

a. Number of bacteria, fungi and actinomyces.

Plate counts on albumin agar and casein $agar^{1}$) were carried out in the autumn of 1941 and of 1942 and in the spring of 1950. These estimations were made in the usual way after shaking the soil samples vigourously in tap water for 5 minutes and incubating the agar plates for 5 days at 25° C.

From these figures it will be seen that liming of the acid peat soil has stimulated the development of actinomyces to a much higher degree than that of bacteria. In 1942 for instance bacteria numbers were nearly equal in the acid and neutralized soils whereas actinomyces counts of the neutralized soil were eight times as high as those of the untreated soil. In 1950 bacteria counts were also considerably higher in the limed soil but in this case also actinomyces had increased about twice as much.

¹) These media contained glucose I g, K_2HPO_4 0.5 g, $MgSO_4$. $7H_2O$ 0.2 g, $Fe_3(SO_4)_3$ trace, agar 20 g, tap water 1000 cc, and 0.25 g of albumin and 1.0 g of casein dissolved in 8 cc of 0.1 N NaOH, respectively.

This increase in development of actinomyces upon liming was reflected in a large increase of potato scab.

| CaCO ₃ , applied in 1941, tons per ha | kg N/ha as NH₄NO ₃ | autumn of 1941 | | | auti | umn of | 1942 spring of 1950 | | | | |
|--|-------------------------------------|----------------|------------------|-------|---------------|------------------|---------------------|---------------|------------------|-------|--|
| | | bac- teria | actino- myces | fungi | bac- teria | actino- myces | fungi | bac- teria | actino- myces | fungi | |
| | o | 0.8 | 0.6 | 0.I | 6.3 | 0.7 | 0.4 | 4.0 | 0.9 | 1.1 | |
| 0 | 60 | |) — ' | | 7.4 | 0.7 | 0.5 | 4.9 | 1.4 | 1.0 | |
| 2 | 0 | — | - | | | | | 5.4 | I.4 | 0.9 | |
| 2 | 60 | — | — | | | — | | 5.6 | 1.5 | 1.1 | |
| 5 | 0 | | — | | — | — | | 5.9 | 1.7 | 0.8 | |
| 5 | 60 | | — | | — | — | | 6.0 | 1.3 | 0.8 | |
| 10 | 0 | 1.2 | 17 | 0.I | 7.7 | 1.9 | 0.4 | 5.9 | ĭ.4 | 0.5 | |
| 10 | 60 | — | | | 6.1 | 2.6 | 0.5 | 5.2 | 2.3 | I.I | |
| 30 | 0 | | I — ∣ | | | — | | 10.6 | 3.4 | 0.8 | |
| 30 | 60 | | I — | | | | | 8.2 | 4.3 | 0.5 | |
| 60 | 0 | 2.4 | 3.8 | 0.I | 8.r | 6.0 | 0.4 | 10.2 | 4.9 | 0.6 | |
| бо | 60 | — | - | | 7.0 | 5.5 | 0.5 | 16.9 | 5.6 | 0.2 | |

TABLE 2. Millions of bacteria, actinomyces and fungi per one gram of moist soil.

Since actinomyces are capable of attacking less decomposable organic matter, it is very likely that increase of plant-available soil nitrogen after liming has been due mainly to the activity of these organisms.

b. Azotobacter.

The presence of Azotobacter was tested by adding I g of the soils to ER-LENMEYER flasks of 300 cc capacity containing 40 cc of a nutrient solution of the following composition: glucose IO g, K_2HPO_4 I g, MgSO₄.7H₂O I g, CaCO₃ IOg, Na₂MoO₄. 2H₂O 0.5mg, FeCl₃. 6H₂O IO mg, ZnSO₄.7H₂O I mg, tap water IOOO cc, and incubating at 25° C. No positive Azotobacter test was obtained in the first two years after liming. Apparently Azotobacter was absent from this soil and was not introduced into it after neutralization during the first few years. In 1950 positive tests were obtained with soil from plots treated with 60 tons of calcium carbonate in 1941.

c. Nitrifying organisms.

Activity of nitrite and nitrate-producing microorganisms was determined by adding I g of the soil to ERLENMEYER flasks containing 20 cc of a nutrient solution of the following composition, *nitrite-producing bacteria*: $K_2HPO_40.5g$, $MgSO_4.7H_2O0.5g$, $Fe(NH_4)_2$ (SO_4)₂.6H₂O0.25g, CaCO₃ IO g, (NH₄)₂SO₄ (added after sterilization of the solution) I g, tap water 1000 cc. *Nitrate-producing bacteria*: $K_2HPO_40.5$ g, $MgSO_4.7H_2O$ 0.3 g, Na_2CO_3 I g, NaCl 0.5 g, FeSO₄.7H₂O 0.4 g, NaNO₂ I g (added after sterilisation of the solution).

Rate of nitrite formation and of ammonia transformation were used as a measure of the activity of nitrite-forming bacteria whilst activity of nitrate-forming organisms was derived from rate of transformation of nitrite. The results of these experiments are shown in Table 3. It will be seen that a ready transformation of ammonia as well as of nitrite occurs in the solutions inoculated with heavily limed soil. Apparently great numbers of nitrifying bacteria may be found in limed acid soils a few months after liming. Similar results were obtained in the autumn of 1942 and in the spring of 1950.

| CaCO ₃ , applied in 1941, tons per ha | kg N/ha as NH ₄ NO ₈ | N in | litrite-fo | Nitra b in perio | ate-forming acteria, cubation od (weeks) | | | | | |
|--|--|--------------------------------|------------|---------------------------|---|-----------------|--------|-----|-----|-----------------|
| | | I | | 2 | | 3 | | I | 2 | 3 |
| | | NH ₃ ¹) | NO_2^1 | NH3 | NO_2 | NH ₃ | NO_3 | NO2 | NO2 | NO ₂ |
| 0 | 0 | 5 | 0 | 5 | 2 | 4 | 3 | 5 | 3 | 0 |
| 0 | 8o | 5 | 0 | 5 | I | 4 | 3 | 5 | 3 | r |
| 2 | 0 | 5 | 0 | 3 | 5 | 2 | 3 | 5 | 3 | 2 |
| 2 | 80 | 5 | I | 4 | 5 | 3 | 3 | 5 | 3 | , r |
| 5 | 0 | 5 | 2 | 3 | 5 | 3 | 4 | 5 | I | io – |
| 5 | 80 | 5 | 2 | 2 | 5 | I | 4 | 5 | 0 | 0 |
| 10 | 0 | 5 | 2 | r | 5 | 0 | 2 | 5 | 2 | 0 |
| IO | 80 | 5 | 3 | 2 | 5 | r | 4 | 5 | I | 0 |
| 30 | 0 | 5 | 3 | I | 5 | 0 | 2 | 5 | 0 | 0 |
| 30 | 80 | 5 | 4 | 2 | 5 | I | 0 | 5 | 0 | 0 |
| 60 | 0 | 5 | . 4 | I | 5 | 0 | 3 | 5 | 0 | 0 |
| 60 | 80 | 5 | 4 | I | 5 | 0 | 0 | 5 | 0 | 0 |
| | • | | | | | | | | | |

TABLE 3. Effect of liming of an acid peat soil on activity of nitrifying bacteria. (October 1941).

¹) qualitative tests; NESSLER's reagent and GRIESS-ROMIJN's reagent were used respectively; 5 = strongly positive, o = negative test.