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INVESTIGATIONS ON THE NITROGEN NUTRITION OF PLANTS

I. A NEW METHOD FOR THE DETERMINATION OF NITROGEN-REQUIREMENT OF SOILS

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I. *Introduction.* In the determination of fertilisation needs of soils, nitrogen causes the most difficulties, as only a small and very variable part of the total nitrogen in the soil can be used directly by plants. Even for the other basic nutritional elements, potassium and phosphorus, total content in the soil does not always indicate the availability of these elements for plants. Most of the K and P is not readily available for uptake by plants, but must first be liberated by weathering of soil particles or by the dissolving action of excretions from the plant-roots. It may consequently be looked upon as an equilibrium between the absorbing action of the soil and the dissolving action of the roots.

Regarding nitrogen, however, the situation is fundamentally different. The total stock of this element in the soil is almost entirely restricted to organic substances, either stabilized humus or the still undecomposed particles of vegetable or animal origin. In these organic substances nitrogen occurs in the proteins and in protein-like compounds. By the attack of mineralising microbes, small amounts of this protein nitrogen are continually transformed to inorganic forms — ammonium compounds, nitrites and nitrates. The nitrogen cannot become available for the plants before this disintegration, because the proteins and related compounds cannot be assimilated by the roots. The nitrogen in the soil may thus be divided into two fundamentally different fractions: the bulk of the nitrogen in the unavailable form of organic compounds, and the comparatively much

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smaller part, comprising the mineralised nitrogeneous compounds, all of which can be almost entirely absorbed by the plants immediately. It is true, that of this mineral nitrogen, the ammonium compounds can be absorbed by soil particles, but quantitatively the NO_3-nitrogen in most soils is of much more importance than the NH_4-nitrogen. Besides, the absorbed NH_4-ions are just as readily available for the nutrition of plants and microbes as the nitrogen in solution.

Development of most of the annual crops in the temperate-climate zones starts at a time when the content of mineral nitrogen in the soil is relatively high, since late spring is the period of the highest mineral nitrogen level in the soil as a result of comparatively active mineralisation in the warm days of spring without any appreciable uptake of nitrogen by the very young plants. But this stock of mineral nitrogen can never become very large. During the preceding winter and early spring, the temperature was too low for active disintegration of the humus, and rainfall surpassed evaporation, while the period between improvement in the climatic conditions and the start of plant growth is too short for an accumulation of appreciable amounts of mineralised nitrogen. Numerous reports in the literature as well as our own investigations confirm this observation: it is seldom that more than 20 to 30 mg of mineral nitrogen per kg of dry soil is found in ordinary arable soils at that time of the year. Thus, without an intentional fertilisation, only the first part of the development of a crop can be supported by accumulated nitrogen, while as soon as the crop comes into the stage of its most rapid growth, it starts to absorb so much nitrogen that this absorption surpasses in most soils the production by the mineralising action of microbes. Consequently the stock of mineral nitrogen, available at the beginning of the vegetation-period, is soon exhausted and the crop for the rest of its growth depends exclusively upon the nitrogen liberated by microbes from the humus. This is most pronounced for all types of perennial vegetation — pastures, grasslands, forests and wintercereals. For these even the short period of nitrogen-accumulation in the spring may be levelled off by the early starting growth of the plants.

On the other hand, during the warm summer-season, mineralisation of the nitrogen can become very significant in fertile soils, producing, sometimes within a few weeks, more assimilable nitrogen than the above mentioned stock, accumulated during the spring.

Once this scheme of nitrogen-nutrition of the plants is accepted,

the inevitable conclusion follows, that determination of the amount of any form of nitrogen in the soil cannot give a correct estimate of fertilisation requirements. Only a measurement of the activity and the rate of mineralisation of the nitrogen containing organic matter in the soil, can serve as criterion for this purpose. The limitations of the value of nitrogen determinations become still more apparent when we consider that sometimes even during the summer season the weather-conditions can bring about very rapid and very considerable ups and downs in the nitrogen content. After a period of dry sunny weather, permeating the soil with innumerable cracks, one heavy thunderstorm or a succession of rainy days can deprive the top-soil of nearly all the available mineralised nitrogen.

It is therefore by no means surprising that none of the many proposed methods for the determination of available nitrogen in the soil proved to give correct insight into the fertilisation requirements. It is true, that the determination of the amount of nitrogen, when compared with practical observations of crop development over a long series of years, can give some indications concerning the nitrogen requirements of the soil. Sometimes it is even possible to determine values of nitrogen content corresponding with certain yields of the crops. But these values can never be generalised, since they are valid only for the soil and for the conditions where the study was done. Consequently it was gradually recognised that the value and reliability of such investigations are rather disappointing. G i e s e k e [3]) a.o. when speaking about the determination of the total nitrogen content of the soil, summarised this opinion by saying: .... „Diese Feststellung ist vereinzelt, so das die chemische Bestimmung des Bodenstickstoffs in ihrer Geeignetheit zur Ermittlung des Düngebedürfnisses der Pflanzen als fragwürdig erscheinen muss"..... And discussing the determination of the mineralised nitrogen of the soil .... „Doch selbst die Bestimmung der löslichen N-Verbindungen ist für die Ermittlung der N-Bedürftigkeit wohl kaum von grosser Bedeutung, da die im Boden vorkommenden Mengen nicht nur sehr klein, sondern auch sehr schwankend infolge der immerwährenden Umsetzungen sind"..... These opinions became gradually more and more uniformly accepted around 1920, whereby the high expectations of the early days of agricultural research seemed to be destroyed without finding another way to reach the goal.

The first sign of finding a way out in this dead-lock was the intro-

duction of the determination of the nitrogen, available for plants in the soil, simultaneously with an analytical determination of the total nitrogen content of the soil. Through these investigations it was soon recognised that the C : N ratio in the soil was important, and consequently efforts were started to detect the nitrogen requirement of soils from the determination of their C : N ratio in addition to that of their total N-content. But before long it was again realised that the mineralisation of nitrogen depends on more factors than these two alone. Thus conclusions derived from such investigations were still unreliable.

Purely empirical methods for the determination of the availability of nitrogen had no more success: the method of M i t s c h e r l i c h, the analysis of mature plants or seedlings (method of N e u b a u e r) or the determination of the NO_3-content in plant-juices as proposed by N i k l a s and V o g e l. The first-mentioned method became a failure when applied to nitrogen since the action-coefficient of N is very small. Consequently very much nitrogen had to be used, resulting in severe lodging of the crops. Moreover this determination is very vague as a result of the very rapid changes in the amount of the available mineralised nitrogen. All methods based on the chemical analyses of plants are handicapped by the sometimes very significant surplus-adsorption of nitrogen by plants, giving a complicated and varying relation of the growth to the quantity of absorbed N, so that it proved to be impossible to use thise determinations for a reliable evaluation of the nitrogen requirement of soils.

Better results were unattainable until the first attempts were made to determine the ammonifying and the nitrifying capacities of the soils, especially if these determinations, instead of being made in a liquid medium inoculated with the soil concerned ,were done directly in the soil, with or without (but preferably without) additions of proteins in the first case and ammonium compounds in the second. These ammonification and nitrification determinations were the first step on the right way as they introduced for the first time the principle of keeping the soil samples for a certain period in favourable conditions before analysing for ammonia and nitrate compounds, thereby giving the microflora an opportunity to achieve a certain mineralisation of the humus. One of the first reports about such determinations was made by B o g d a n o w [1]) as early as 1900, when he described very elaborate and perfected experiments of this

kind. Many years later these methods were improved and standarised still more by N ě m e c [5] [6], but the best modification of these methods was described by V a r a l l y a y [7] in 1937.

Yet even these methods did not give the expected clear information. Their first shortcoming is that they concern only one stage in the whole cycle of mineralisation of the humus nitrogen. But the fact that the amount of nitrates and ammonium compounds at the time of sampling is not determined and thus not taken into consideration is much more serious, because this initial concentration of the mineral N proved to be of primary importance in regulating the course of mineralisation, as will be discussed later in detail. Therefore it is even insufficient to determine analytically the concentration of mineralised nitrogen in a soil at the time of sampling for an ammonification and nitrification experiment. Really comparable results can only be obtained when starting on the same very low level of mineral N in the samples. It is even surprising that it was possible to obtain some satisfactory results with the application of such ammonification determinations. This can presumably be explained, considering that very often the sampling is done in summer, when most soils are practically exhausted by the crops and have very small quantities of mineral N. In rather poor soils such periods of very low levels of mineral N may occupy practically the whole year, since in such soils only twice a year, in late spring and early autumn the mineral N-content is increased. Generally speaking, these ammonification and nitrification experiments cannot claim to be consistent and correct applications of the sound principle that the nitrogen requirement of a soil can only be determined when the whole course of mineralisation is followed, starting on the level of a very low concentration of mineralised nitrogen.

In the subjoined a new method will be described, based on the same ideas but trying to follow them as consistently as possible.

II. *The principle of the method.* More than 10 years ago, performing ammonification and nitrification determinations in the soils of the recently reclaimed Wieringermeer-polder, it was observed, that the results of these determinations were greatly influenced by soil conditions at the moment of sampling. Comparable and reproducible results could only be obtained when the sampling was restricted to times of similar conditions and with the same level of mineralised

nitrogen in the soils. In all soils, but especially in those with a low humus content, increase in mineralised nitrogen proved to be the more rapid, the lower this amount was at the moment of sampling. The next step was consequently to try to reduce the concentration of mineralised nitrogen artificially, if too high, before starting the determination, by extracting the mineralised nitrogen from the soils. By this treatment the soil samples were put under the conditions prevailing in the soils during most of the year. It now proved possible to get good results also when the amount of mineralised nitrogen in the soil happened to be high when the samples were taken. These results were then comparable with those obtained in periods of very low concentration of mineralised nitrogen. The best results could be obtained, when this extraction was performed not by percolation of the samples by water but by cultivation of a fast growing crop to absorb the N from the samples. Hereby the soil structure was altered less than by the water treatment.

These fundamental observations were sufficient to indicate the right way for the development of a standardised, generally applicable method for determining the intensity of mineralisation of nitrogenous compounds in humus, and indirectly also of the nitrogen requirements of soils. This method comprises the following successive steps: 1. Extraction of available mineralised nitrogen, preferably by placing the samples in shallow containers and sowing them thickly with a fast growing crop. 2. Removal of the crop and transferring of the samples to conditions ideal for mineralisation of the humus. 3. Analytical determinations at regular intervals of the content of mineralised nitrogen in the samples kept under these favourable conditions. 4. Plotting the results in graphs. The course of the gradual increase in mineralised nitrogen, plotted against time, then offers a measurement of the intensity of mineralisation.

Fundamentally this method can be divided into two periods: the *extraction-period* followed by the *period of regeneration* of the stoc of mineralised nitrogen removed by the extraction. During the regeneration period the samples must be kept under very constant conditions, accurately standardised, to assure sufficiently comparable results.

It must be emphasized that, in this method, the maximal amount of mineralised nitrogen finally reached is not an indication of availability of nitrogen for plants, but rather the rate of increase of this

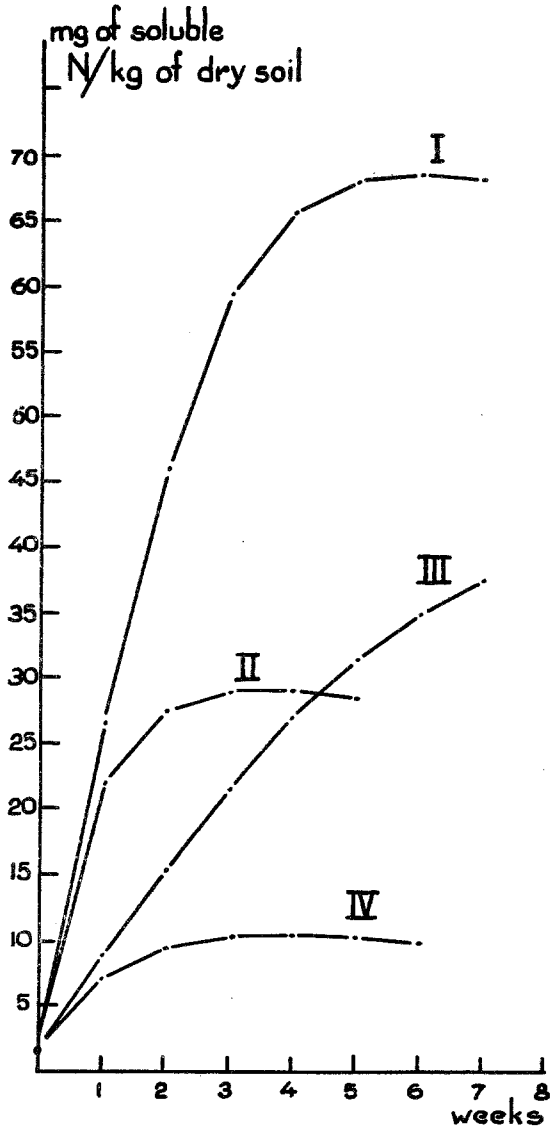

Fig. 1. Diagrammatical, "ideal" curves, indicating the mineralisation of nitrogen.

I: Soil ensuing a very rich nitrogen-nutrition of the plants. A curve with a very steep slope, running up to a high level.

II. Soil giving a moderately good nitrogen-nutrition. A curve with a steep slope, but bending over at a low level within two weeks.

III: Soil giving a poor nitrogen-nutrition. A flat curve, but running up to a comparatively high level. Under favorable conditions a rather high level of mineralised nitrogen can be formed.

IV: Soil with a very poor nitrogen-nutrition. A flat curve bending over to the horizontal within two weeks at a very low level.

Soil II supplies the plants decidedly better by nitrogen than soil III, since the slope of it is much steeper during the first few weeks.

amount during regeneration, especially during the first part of the regeneration. It is this rate and not the final level of mineralised N which represents the quantity of absorbable nitrogen, provided to the growing plants in a unit of time by the soil.

The best method of assembling the analytical results obtained from repeated determinations of soluble nitrogen during regeneration is without doubt to plot them in a graph against time. The possible types of curves obtained by doing so are diagrammatically shown in Fig. 1.

Apart from the principle on which the whole method is based, but nevertheless very important, was the question whether the level of the whole mineralised nitrogen can be represented by the determination of only one part of it — the nitrates. It is true that the nitrates in most soils are by far the most important constituent of the whole complex of the different forms of soluble nitrogen, and also that they belong to the most readily absorbed part of it, but a more detailed study of this problem led us to the conclusion, that even in soils under ordinary conditions, the ammonia compounds and sometimes also the water-soluble organic nitrogenous compounds (amino acids, urea and others) may not be disregarded. An illustration of the figures on which this conclusion is based is given in the data brought together in Table I.

These figures are selected from a large number of similar determinations and they can be backed by analogous data from different publications in the extensive literature concerning this subject, but they are sufficient to show that while in most cases the NH_4 and organic components in the total amount of soluble nitrogen are really very small, sometimes, especially when dealing with permanent grasslands or peaty soils, they are much larger, representing such high percentages that they cannot be disregarded. The determination of the nitrogen available for plants, based on analysis of the nitrates only shall therefore always be a rather uncertain method. Moreover, the process of nitrification and the last phases of disintegration of the soluble organic compounds are very fast, consequently the ammonia compounds and the soluble organic nitrogen may be considered as equivalent to the nitrates for the nutrition of plants, independent of the question whether the NO_3-ion really is the most suitable form of nitrogen-nutrition for the plants.

Both these considerations resulted in the decision not to rely upon

TABLE I

Different forms of nitrogen found in clear water-extracts of some representative soils, expressed as mg N per kg of dry soil

Characteristics of the soils (sampled from 0–20 cm deep)	Date of sampling	Nitrogen determined as	
		NO_3	NH_4
1. Heavy clay soil from the Groetpolder, province of North-Holland, arable land, rich in nitrogen, lying fallow	7- 7-1941	20.4	1.3
	4- 8-1941	21.1	0.2
	1- 9-1941	10.7	0.0
	8-12-1941	7.6	0.3
2. The same soil but in clover	7- 7-1941	11.1	0.9
	4- 8-1941	10.6	0.8
	1- 9-1941	17.9	0.4
	8-12-1941	38.0	2.1
3. Light sandy clay near St. Anna-Parochie, province of Friesland, arable land, lying fallow	7- 7-1941	20.3	0.7
	2- 9-1941	11.0	0.3
4. The same soil but in clover	7- 7-1941	8.0	0.2
	2- 9-1941	6.3	0.3
5. Heavy clay-soil in the Province of North-Holland, permanent grassland, that year used for hay-making	29- 7-1942	5.0	2.8
6. Approximately the same soil, permanently used as pasture	4- 8-1941	11.0	2.0
	24- 8-1942	5.6	5.0
7. Boggy soil, slightly acid, very rich in organic matter, near Giethoorn, Province of Overyssel arable land, in potatoes [1])	15- 7-1946	14.3	24.0

[1]) While in the other samples inlcuded in this table NO_3 and NH_4 account for nearly all the water-soluble nitrogen, leaving only negligible quantities for other nitrogenous compounds, in this boggy soil about 3 mg N was found as unidentified nitrogenous compounds.

the determination of nitrate-nitrogen only but to apply a method giving the total nitrogen soluble in water, thus representing the nitrogen immediately available to the plants. In the beginning the Jodlbaur-modification of the Kjeldahl-method was used, but in searching for a quicker and easier method, it was soon recognised that the different modifications of the method of reducing all nitrogen-forms in an alkaline medium by nascent hydrogen were able to give very exact and reproducible results, identical with those of the Jodlbaur-method when checked with the latter. It was also possible to recover quantitatively known amounts of added pure nitrates, ammonia-compounds, urea and amino acids. The hydrogen is evolved by boiling alkali reacting on finaly ground metal alloys. The advantage of these methods is the simultaneous destruction, reduc-

tion and distillation, which makes this method much quicker. From the numerous possible modifications, that using 3% NaOH and the Devarda-alloy was selected in a slight modification. This method proved to be very reliable and, when standardised, to allow very rapid serial work, therefore it was used almost exclusively throughout this whole investigation.

III. *The method.* The taking of the samples does not need to be described here, since no special manipulations are required. Only the usual rules for all similar samplings must be observed. But within these limitations the whole procedure must be improved and refined as much as possible, since the critical factor determining the rate of nitrogen-regeneration is humus, which is an insoluble substance, very irregularly distributed throughout the soil. Therefore sampling and mixing of the samples have to be done with the greatest possible care and the samples must be composed of a sufficiently large number of borings [4]). In most cases the samples comprised 25 or 50 borings, regularly distributed over the entire surface of the plot under investigation. The samples consequently were rather large, sometimes exceding 10 kg since it was impossible to use borers with a small diameter on the wet and sticky soils.

The question, how deep must the samples be taken, cannot be answered here, since that depends on the purpose of the investigation. On regularly ploughed arable land it is advisable to take samples from the entire turned layer. The surface-layer of 2 to 3 cm is often in an unusual condition — it may be crusty or very dry or crumbled. It may therefore be advisable to remove this layer before sampling. On grassland with a tough sod it is immerative that the turf be removed since it would otherwise greatly increase the amount of fresh organic matter in the sample. The disintegration of this fresh organic matter, generally not rich in nitrogen, should appreciably decrease the rate of nitrogen-mineralisation. Even much smaller quantities of such fresh organic matter (especially living thin roots of plants) sometimes causes very confusing deviations from the normal results.

In the mixing and pretreatment of the samples before analyses, it proved to be impossible to mix the large primary samples by a simple moulding by hands or scoops, especially when the soil was wet and sticky or contained stiff lumps. Yet this mixing

had to be done with much care in fiew of the irregular distribution of the humus in the soil [4]). Consequently all the large heavy samples had to be taken home for mixing and reducing to the required size by special crumbling and distributing apparatuses. The apparatus for crumbling the samples was designed and built especially for this purpose as a rather rough but strong blacksmith construction. It is diagrammatically shown in Fig. 2.

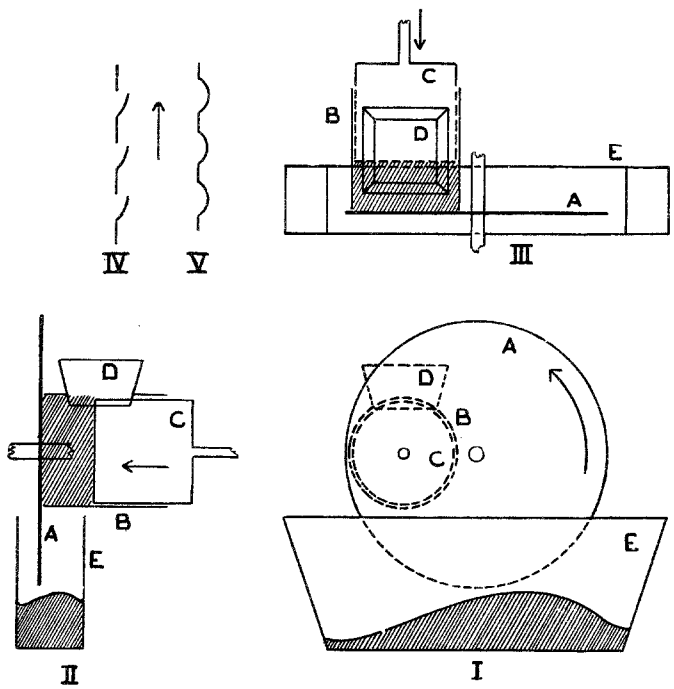

Fig. 2. Digaram of the apparatus for crumbling the soil samples.
I: Side-view.
II: Vertical cross-section.
III: Plan.
IV: Radial cross-section of the disk on a larger scale.
V: Tangential cross-section of the disk on a larger scale.
The lever for moving the piston and the handle for revolving the disk are not shown in the diagram.

The most essential part of this apparatus is a strong round iron disk revolving in a vertical position (*A*). The disk is perforated by a large number of notches of the same type as in a rough soap-grater (See Fig. 2 IV and V). It revolves in front of and very near the outlet of a horizontal, hollow

cylinder (*B*). In this cylinder a piston (*C*) can be moved by a lever. Through a funnel-shaped opening on the upper side of the cylinder (*D*) the soil can be poured into it. Then simultaneously the piston is pushed in by the lever and the disk is revolved. Hereby the soil is pressed against the disk and the notches scrape thin shavings from the soil. These shavings are then collected in a trough (*E*) underneath the disk. It was possible in a short time to transform even the stickiest and thougest soils to a crumbling mass.

From the crumbled primary sample, small samples can now be taken for the determination of moisture and mineralised nitrogen content at the beginning of the whole procedure. The most adequate method for this secondary sampling is the use of a so called "sectorial divider" [4]), which produces a completely reliable sampling when the material is sufficiently finely crumbled.

The sample is now poured into a shallow container and densely sown with a fast growing crop, which absorbs much nitrogen in a relatively short time. The best results could be reached using ordinary spinach (*Spinacia oleracea* L.). As containers, the shallow unglazed earthenware dishes used by horticulturists for nursing of seedlings proved to be adequate. The depth of these dishes is only about 8 or 9 cm; thus while spread out in a comparatively shallow layer, the sample is quickly extracted but it suffers from very rapid evaporation. It is thus necessary to restore the original water content frequently by watering. Since the dishes are made from porous unglazed earthenware, the formation of waterlogged anaërobic corners in them is unlikely. These dishes should not be deeper than 10 cm because the depth of the soil may not exceed about 8 cm, and high edges above the soil would cause shadow-strips and weak growth of the spinach. The minimum size of the dishes must be just sufficient to contain enough soil for the whole series of analyses. Since in our investigation all determinations of mineralised nitrogen were made as macro-analyses, each determination required 300 g while a complete series comprised 6 or 7 determinations. Thus the samples had to be at least 2.5 kg. But to prevent abnormal conditions in the last sub-samplings, the minimum weight of the samples had to be fixed at 5 or 6 kg. Very adequate proved to be the size of these earthenware dishes with a capacity of about 7 kg of soil. However, during the war and the first post-war years it was impossible to get such earthenware dishes. They had to be replaced by small wooden cases of approximately the same shape — a very bad

substitute. Under the conditions of regeneration of the mineral nitrogen in the thermostate, high temperature and moisture caused a very rapid decay of the wood by wood-rotting organisms, and the growth of these organisms was only possible when part of the mineralised nitrogen released in the soil was absorbed by them. The result was thus a delayed and disturbed mineralisation. This shortcoming of the wooden cases became more and more apparent when they were used for a long time. Therefore each case could be used only 2 or 3 times, before the results became too much affected by the decay of the wood. At the laboratory of Dr. C r o w t h e r at the Rothamsted Agricultural Experimental Station, round dishes of glass are used for a similar purpose (unpublished oral information). No difficulties resulting from anaerobic conditions near the bottom have been observed at Rothamsted in these impermeable containers. It may therefore prove to be possible to alter the whole procedure to micro or semi-micro analyses and consequently also to much smaller samples and containers. But micro analyses require more carefully mixed and homogenised samples. It will then therefore be necessary to change and to improve the crumbling and mixing of the samples.

To ensure a quick and regular germination of the spinach, the seed must be distributed very regularly over the whole surface and must then be covered by a layer of the soil approximately 1 cm thick. When earthenware dishes of approximately 30 × 30 cm are used, the best quantity of good germinating seed for one dish is about 700 grains of seed (approximately 15 g).

During the summer season the light-intensity is sufficient for raising healthy spinach-crops in a hothouse; only the usual measures of ventilation, prevention of burning during the hot hours, and watering must then be taken. But in winter-time a satifsactory development of the crop is only possible when artificial irradiation is applied. The usual Neon-light irradiators are most succesfull. In emergency cases, ordinary lamps can be used, but the light-intensity must then be very high. It is not necessary to give here more details about the technique of raising the crop and its irradiation in winter-time, since no special measures have to be taken and the general rules for hothouse culture can be followed.

Another very important detail, that must be mentioned in connection with this culture, is the danger of epidemic diseases in the spinach especially in winter-time when ventilation in the hothouse is

always lacking. All generally recommended measures were taken to prevent or suppress such epidemics, but it was not always entirely possible to suppress development of mildew or of *Botrytis*-blight.

Since in preliminary experiments it was observed that, in most soils, the natural amount of K and P was insufficient for an optimal development of the spinach or for an optimal mineralisation, it was considered safe to add these elements artificially at the moment of sowing the extraction-crop. An amount of 0.2 g K_2HPO_4 per kg of soil was always sufficient to abolish this deficiency.

As a rule it is not difficult to determine when the crop has taken up all available mineralised nitrogen. The rapid growth of the thickly sown crop ensures a rapid and complete exhaustion of the mineral nitrogen, and that moment is unmistakably marked by a yellow discoloration, cessation of growth and premature flowering of the spinach, all characteristic symptoms of N-deficiency. Therefore it does not require much training to be able to estimate the time of complete exhaustion rather accurately. The whole progress of the growth and the yellowing of the crop are as a rule so demonstrative, that it can often be used for a rather accurate prediction of the amount of mineralised nitrogen and of the intensity of mineralisation since exhaustion is reached sooner when the mineralisation is weak. In some exceptionally N-rich soils, as for instance some rich gardensoils and some marine deposite, exhaustion cannot be reached by one crop of spinach, and the sample must be sown twice for reasonable exhaustion. On the other hand, in very N-poor soils, complete exhaustion can be reached within 3 or 4 weeks, but in normal soils a vegetation-period of 5 to 6 weeks is required.

At the end of the exhaustion period it is not sufficient to remove the crop by pulling it out. Too many fine roots would then remain in the soil and they should cause too much nitrogen uptake during their decay. A careful sieving of the whole soil with simultaneous removal of all visible rootlets is necessary. The easiest way to proceed is to shake off the soil gently from the roots of the plants above a sieve, holding them by their top ends and trying to prevent the breaking of the rootlets as much as possible.

Before the carefully sieved and mixed sample is replaced in the dish, small samples are taken from it for the determination of moisture and mineral nitrogen content at the start of the regeneration period since the exhaustion of the available nitrogen is never com-

plete. There seems to be a minimum limit of concentration in the soil, which the plants cannot remove even if the exhaustion period is prolonged. This minimum quantity varies in different soils. But when the exhaustion is performed properly, there is never more than 3 mg of water-soiuble N per kg of dry soil left. Therefore this limit was adopted as a critical maximum allowed at the beginning of the regeneration.

The moisture content of the samples must be adjusted at this time to approximately 60 to 70% of the total moisture-holding capacity of the type of soil concerned.

For the regeneration-period the samples are brought in the same earthenware containers to a dark thermostatically controlled room, where a temperature of 29°C. is maintained and the atmosphere is approximately saturated with water. To save space, the dishes are put on racks extending from floor to ceiling, leaving only narrow interspace between the successive shelves. Because of this arrangement, it is strictly necessary to operate a small fan in the room, to attain regular distribution of temperature and moisture over the entire space. Maintenance of the very high humidity in this room is essential. Only in an atmosphere practically saturated with water, can evaporation from the soil be reduced to a negligible level, which renders any watering of the samples during the regeneration period superfluous. It must be strongly emphasized, that it is strictly necessary to reach so high a humidity since this is the only way to obtain a really undisturbed process of regeneration. It is useless to place open dishes filled with water in the room. Even a continuous dripping of water proved unsuccessful. The only effectual measure was the evaporation of water at a higher temperature than the average temperature in the room. For this purpose the following arrangement was designed and proved to operate satisfactorily: Heating of the room was performed by electric coils placed in the lower end of a wide pipe, fixed in a vertical position in the room, and open at both ends. At the top end of this pipe a small fan was fixed so as to maintain a continuous upward air flow through the pipe. Halfway up the pipe a thick piece of cloth was stretched in an inclined position and water from a perforated pipe-line was continuously dripping on the cloth, keeping it soaked, while warm air from the electric coil passed along it and caused a very rapid evaporation. By a proper regulation of the distance between the heater

and the cloth it was possible to adjust the evaporation accurately to ensure a nearly complete saturation of the atmosphere.

For the regularly repeated sampling of the soil during the regeneration period the same procedure was followed as at the start of the regeneration. The whole amount of soil in the container was mixed and sampled using the sectorial divider. The rest was then replaced in the container. Usually samples were taken once a week. For special studies sometimes shorter intervals were adopted.

These periodical samples had to be analysed for their moisture and mineralised nitrogen contents. About the first mentioned analysis no details have to be given since the ordinary methods were followed, but for the nitrogen analysis a complete description must be given because a method was applied in this investigation which deviated slightly from the standard prescriptions given in handbooks:

250 g of the soil (with the natural moisture content) are rotated for 2½ hours at room temperature on a shaker in a flask of 1000 ml volume, with 600 ml distilled water and 3 g of $CaSO_4$ (free from N) to aid a rapid and clear precipitation after the shaking. Then the soil was allowed to settle overnight. If insufficient clear solution was available above the precipitate, the whole flask had to be filtered. 300 ml of this clear solution was pipetted into a distillation flask of 1000 ml, 30 ml of a NaOH-solution of 30% by weight and 3 gr Devarda-alloy were added and the flask immediately connected to the cooler on the distillation-stand. The Devarda-alloy must be finely ground, to give a powder, that passes through a sieve with threads that are 0.17 mm apart. The composition of the Devarda-alloy is 59% Al, 39% Cu and 2% Zn. Before starting the actual distillation with heavy boiling, the flask must be heated very gently for 30 min. on a small flame of not more than 2 cm high. The flame may not be increased before the end of this period. The condensate is collected in an excess of 0.01 n H_2SO_4. The excess acid is titrated back by 0.01 n NaOH. The most succesful indicator used was that of Tashiro (4 parts 0.1% methylred and 1part 0.1% methyleneblue by volume, both as alcoholic solutions). Nearly as good was the mixture of 3 parts of 0.1% bromocresolgreen and 1 part of 0.2% methylred, also as solutions in 95% ethanol.

The determined contents of mineralised N can now be calculated as mg per l of water in the soil or per kg of dry soil. Both ways have advantages and disadvantages. In the first case the figures represent the concentration of N available to the plants, but this value fluctuates with the very variable amount of moisture in the soil. The second method of expression gives better comparable figures, equivalent to

the amount of available nitrogen per acre. But in soils with extreme moisture-holding capacity, as in the peaty soils, these figures may represent much lower concentrations than in ordinary soils, thus claiming better nitrogen-nutrition of the plants than really is offered in those soils.

IV. *The validity of the method and its value.* Although this method, which is based on the principle that provision of nitrogen to plants depends primarily on the course of the mineralisation of the humus and not on the amount of soluble nitrogen available at a certain moment in the soil, tries to follow this principle as strictly as possible, it cannot claim to be perfect. On the contrary, more than one shortcoming could be anticipated and still others were met when the experimental work proceeded. The most important defects will be discussed now.

A. Artificial conditions in the soil during regeneration. Conditions in the samples during exhaustion by the spinach as well as during regeneration of the mineralised nitrogen are not the natural conditions prevailing outdoors in the fields, but are considerably changed and entirely artificial. During the growth of the exhaustion crop the conditions had to be chosen to be ideal for a good and quick development of the crop. They are thus approximately the same as are maintained in all moderately heated hothouses of commercial growers. But during the regeneration period, not attempt was made to approximate natural conditions. On the contrary, the aim was to establish ideal conditions for an optimal mineralisation (crumbled, loose structure of the soil, optimal temperature and moisture content, very good aëration). Thus this method does not yield information about mineralisation as it is found in the soil in situ during the vegetation period, but about the potentially optimal mineralisation in the soil concerned. That is in some respects a severe shortcoming of the method, which it has in common with all methods using mixed samples for the determination of any metabolic activity of the soil. The information given by such methods cannot be directly applied to field conditions and must always be checked by field-trials or by statistical comparison with the results of practical farmers. But otherwise these methods give comparable results, which can scarcely be gained by the determination of the same property in the field, where discontinuity and local

variability of the soil interferes with the accuracy of the results. A reasonable accuracy of the field methods can therefore only be reached by increasing the number of multiple determinations to such a high extent, that the method becomes very laborious and expensive.

All methods using mixed samples, kept under artificial laboratory-conditions, are especially valuable for serial work but are much less valuable for fundamental research. And even for serial investigations the data obtained must be checked by comparations with the results of experimental plots or with special fieldmethods.

B. Influence of physical conditions of soil before sampling on the regeneration. While this method was designed as a laboratory method and all shortcomings of such methods have been accepted as inevitable, a zealous attempt was made to obtain really comparable and reproducible figures. However, in one respect this proved to be very difficult. Even the most carefull crumbling and mixing of the samples was insufficient to standardise the structure of the soil and the conditions for development of the microbes. The results obtained were always influenced by soil conditions in the field at the moment of sampling. This influence may last through the whole procedure of the determination. Such differences can be rather significant between samples taken under dry weather conditions in summer and those taken from the same plot in winter, when the soil is wet, uncovered by the crop and when the microbial activity is low. In Fig. 3 such a difference is shown for a heavy clay from a plot near Kampen, province of Overyssel. The two curves give the impression of belonging to two entirely different soils, but in reality only temporary changes in the conditions caused the marked difference.

The influence of the physical conditions of the soil may be demonstrated still more spectacularly by the curves reproduced in Figs. 4 and 5. Fig. 4 shows the regeneration-curves, both representing the same soil sample, crumbled and mixed as usual. One part of this sample was exhausted by spinach, the other part was percolated by water until approximately the same level of soluble nitrogen was left in it as in the first part; then it was partly dried and crumbled again. Then both parts were simultaneously placed in the regeneration thermostate. It is evident that the percolated part of the sample, that suffered very much in its structure and was badly pepti-

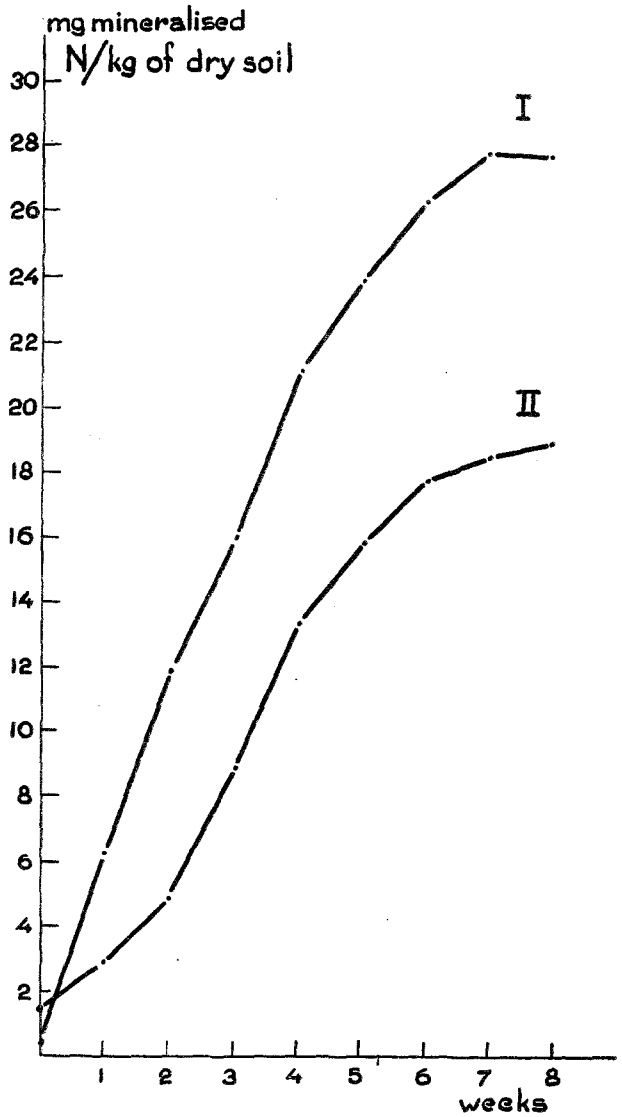

Fig. 3. Curves obtained from samples of the same plot, performing the determination under strictly comparable conditions.

I: Sample taken in August during fair weather. The soil was dry and had a crumbly structure (moisture content 17.2%).

II: Sample taken in December during a rainy period when the soil was practically water-logged (moisture content 34.7%).

sed during the treatment by water, had not overcome this damage during the entire period of regeneration. Macroscopically no much difference was visible between the two parts of the sample, but the internal structure of the soil crumbs remained different, consequently giving also very different rates of mineralisation. It proved to be easy to reverse this result by using the same two parts of the sample

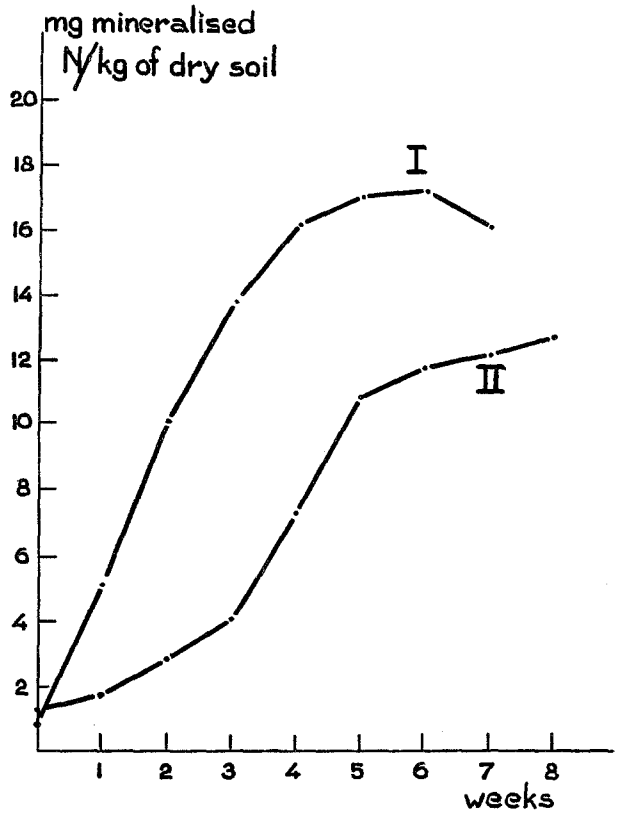

Fig. 4. Curves simultaneously obtained from one soil sample, devided in two parts.

Part I: Treated as usual by cultivating spinach on it.
Part II: Percolated by water.

for a second exhaustion and regeneration, but now using spinach for the part that first was leached by water, and percolation for the part that in the first experiment was sown to spinach. The curves obtained

are shown in Fig. 5. It is evident that the results of the regeneration are now exactly reversed.

Such marked differences as are demonstrated in both the abovementioned examples are found only in soils with a poor, week structure, but since the percentage of soils with a tendency to peptisation is rather high and smaller differences can be found in every soil, it was decided that samples should be taken only in periods of approximately equal conditions: all during the winter season (November–February), thus in wet and cold weather; or all in the period of the most rapid development of the microflora in the late spring (April

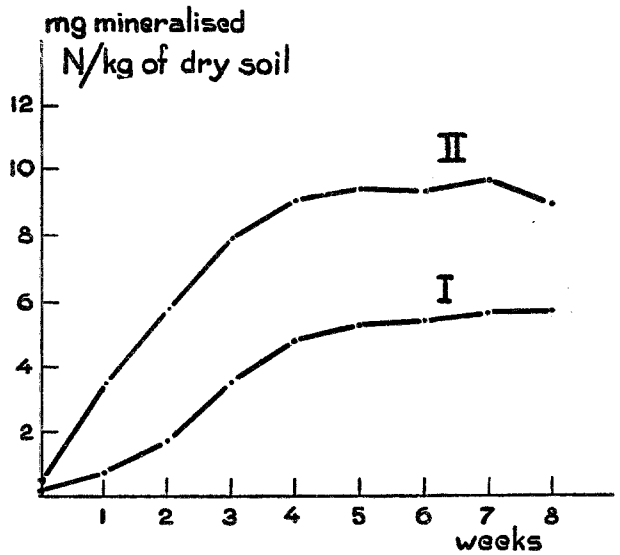

Fig. 5. Curves obtained in a second treatment of the same soil as show in Fig. 4, but with the exhaustion treatment reversed: thus using spinach on part II of the sample, previously leached out by water, and percolating part I, previously extracted by spinach.

and May) before the crops have exhausted the mineral nitrogen in most soils; or all during the second part of the summer when the soils are dry and nearly deprived of their mineralised nitrogen (July, August and September). However, the efficiency of the method is very much reduced hereby and the method is restricted to seasonal periods, which makes it much more expensive and requires a larger staff. For practical application it will presumably be necessary to extend the work over a larger part of the year, taking samples during

two of the three indicated periods or even in all three periods. But the results obtained in different periods must then be corrected to make them comparable. Such a correction will require many comparative analyses on the same soils, sampled in the different seasons. It will be necessary to establish such correction-factors empirically for each type of soil separately. The safest way will be not to use correction-factors found by other institutions for similar soils, but to determine them independently for each experimental station and for each regional investigation before starting the serial work. But it must be emphasized that samples should not be taken during the intermediate periods, i.e., in the late autumn (October) or in the early spring (March), or in June, when soil-conditions are changing rapidly and thus are very inconstant and variable. Also periods of very unusual extreme conditions (the first days after a heavy rainfall, severe droughts, periods of freezing weather and the first days after the thawing of frozen soil, etc.) must be avoided. Such short interruptions in the sampling, however, do not cause serious delays in the procedure of the work, on the contrary they may even be very welcome for overhauling the equipment and to make up arrears in the analytical work.

C. Only the first part of the regeneration-curve can be used for the determination of the nitrogen requirement of the soil. During the numerous determinations performed, using this method, it was always observed that only the first part of the regeneration gives smooth curves, approaching nearly straight lines, while later all curves bend over to the horizontal and become irregular with ups and downs. Only the first smooth and nearly straight part of the curves is therefore suitable for the estimation of the nitrogen requirement of the soil, since it is the slope of the curve in this period of regeneration that indicates the amount of available nitrogen that the plants can get from the soil, per unit of time, under conditions resembling those in the field, i.e. when no appreciable stock of soluble nitrogen is available. But unfortunately this part of the curve is often short, making the whole method less accurate and less reliable, since the slope of this part of the curve then depends on only 2 or 3 consecutive analyses. For some soils the straight part of the curve is longer, but nevertheless even these curves always start turning to the horizontal, simultaneously becoming less smooth, sometimes showing

very sharp ups and downs. The general trend of the curves to approach the horizontal is understandable, although this seeming decrease of the rate of mineralisation is in the nature of the case not really a decrease, since the amount of humus that can be readily mineralised cannot be reduced appreciably within such a short time and also the concentration of the products of metabolism is not nearly high enough to bring about a real decrease of the rate of fermentation. Buth the increase of the concentration of soluble nitrogen compounds gives other groups of microbes the opportunity to attack carbohydrates and other nitrogen-free organic compounds in the humus, synthesizing their own protoplasm. This multiplication of microbes at the expense of nitrogen-free organic compounds and of the mineralised nitrogen is the factor competing with the breakdown of the nitrogen containing compounds of the humus in the release of soluble nitrogen from the organic form. The higher the concentration of the soluble nitrogen becomes, the more microbial protoplasm is synthesized, retarding the rise in the soluble nitrogen-content. Consequently the curves, indicating this rise, turn more and more towards the horizontal during the regeneration.

Less understandable is the tendency of nearly all curves to show pronounced ups and downs in the later phase of the regeneration. These fluctuations indicate some irregular periodicity in the development of microbes and thus also in the uptake of mineralised nitrogen. They are not produced by fluctuations in any of the external growth factors but are spontaneously developed by the microbes, since they occur under strictly constant conditions of temperature, moisture, irradiation, etc. as well as in less accurately regulated cultures. Such spontaneously arising fluctuations are very common in the inorganic as well as in biological processes. Therefore it is not at all surprising that the mineralisation of the humus by microbes also shows very pronounced fluctuations. The ideal smooth curves shown in diagram in Fig. 1 are very rare; nearly always, some irregular ups and downs are observed in the later stages as shown in Figs. 3, 4 and 5. Yet these curves are examples of exceptional regularty. Irregularities in the later parts of the regeneration are usually more pronounced. Typical curves of the latter type are shown in Fig. 6.

Sometimes the ups and downs in the regeneration of nitrogen become perceptible unusually early, in the normally straight part of the curve, making a still greater part of the curve valueless for the

estimation of the nitrogen requirement, and thus reducing the accuracy of the method.

This very pronounced interchange of periods of predominating mineralisation with those of predominant microbial synthesis in the break-down of humus in soil, has already been reported by Gerret-

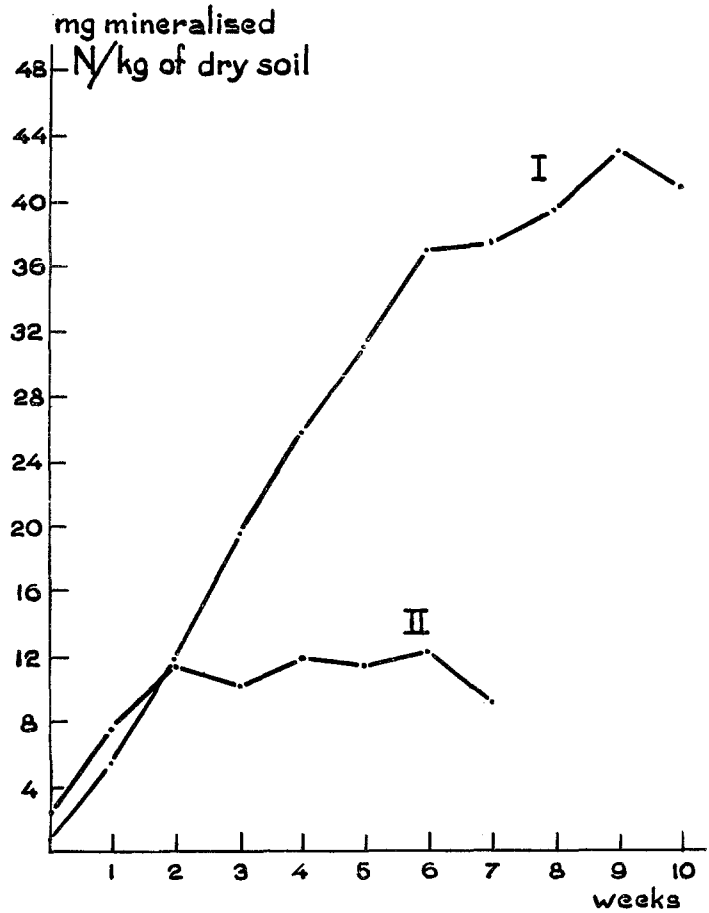

Fig. 6. Two curves of very common types, showing sharp ups and downs in later stages of the regeneration.

I: A steep long curve, characteristic of a very N-rich soil. The irregularities become evident only after 6 weeks of regeneration. A desirable type of curve.

II: A rather steep but short curve, characteristic of moderately N-rich soils. The irregularities disturb the curve after the second week of regeneration, making the determination very uncertain.

s e n [2]) for soil samples kept under similar conditions, without giving a complete explanation of the phenomenon.

D. Analytical determination of mineralised nitrogen is based not upon its availability to the plants, but upon its solubility in water. This is fundamentally a serious shortcoming of this method, as while it is true that all soluble nitrogen is available for the plants, the reverse is not valid. Part of the nitrogen — the NH_4-fraction — that is readily available to the plants is not entirely soluble in water, but is partly retained by the soil-particles as absorbed ions. But practically no troubles were caused hereby in ordinary types of soils in which the amount of ammonia is very low and thus very few ammonia-ions are available among the absorbed bases. Only in the especially ammonia-rich soils, mentioned when describing the method, does this fraction become important, and then it may not be neglected. In those cases, however, it proved to be possible to replace the ordinary water by a solution of *n* KCL or NaCl for the preparation of the soil-extracts for the Devarda-analyses. Hereby the absorbed NH_4-ions could almost entirely be replaced by the Na or K ions and the ammonia consequently was dissolved quantitatively. No troubles were met during the Devarda-analyses of these NaCl or KCl containing extracts.

E. The impossibility of removing the rootlets of the exhausting crop entirely from the soil-samples. At the time of sampling, sometimes the soil already contains many living rootlets, especially samples taken on grassland. But even more trouble is brought about by the rootlets of the spinach, since they are still living when the regeneration is started, while the roots of the original vegetation in the field are already partly decayed during the exhaustion period. Such undecayed fresh plant-tissue, that usually has a rather low nitrogen content, exerts the usual action of all N-poor organic material: in the first stages of its desintegration it absorbs much of the soluble nitrogen from the soil, reducing hereby the rate of release of the nitrogen from the humus. It is therefore quite understandable that the amount of fresh roots in the sample has a pronounced influence on the shape of the observed curves. For a correct operation of this method it is therefore essential to remove the small rootlets of the spinach completely before starting the regeneration. But, as already

mentioned, it proved to be impossible to do this completely. In very loose, sandy soils it was possible to remove nearly all rootlets if the sieving was done very accurately, but the heavier the soil is, the more of the very fine rootlets remain in the samples. Generally speaking, the heavier a soil is and the more fine roots were produced by the crop, the more is the mineralisation thus retarded. To date insufficient work has been done to establish quantitatively the necessary corrections for each type of soil, but this will have to be one of the most urgent measures for an improvement of the method, since now the heavy soils are always appreciably handicapped, compared with the sandy soils. But even when corrections for the main types of soils have been determined, they will always be only an approximation of the influence really exerted by the roots in each special case. Therefore these rootlets remaining in the samples constitute one of the most serious imperfections of the method, and no way to overcome it has yet been found.

F. The method is relatively laborious and expensive. This disadvantage of the method must be mentioned. It is true that it is much easier and cheaper to determine the nitrogen requirement of soils by this method, than by field trials and by collecting the experience of the farmers over many years, but compared with other analytical determinations in soil samples, the method outlined in this publication decidedly is one of the most laborious. The exhaustion of the soil by the spinach requires approximately 5 weeks, while the regeneration takes 6 to 7 weeks. The total duration of the determination therefore is at least 3 months Only when the mineral nitrogen content at the moment of sampling is very low, the especial exhaustion by spinach in cases of emergency may be left out, but for the sake of comparability it is never advisable to do that.

The costs of the total determination are especially raised by the necessity to have a hothouse with heating and irradiation arrangements and of a thermostatically regulated room with humidity control. But the required man-power is also very high, as each sample requires at least seven successive analyses. Until now insufficient serial work has been done with this method to be able to estimate the expenses per sample. The only experience, that could be gained was when the nitrogen requirements of the soils in the SW part of the Netherlands were studied in the years 1945–1947 after the recovery

of the land from flooding by sea-water during the war. But the experience collected there cannot give good information about the expenses, since the conditions for the work were very primitive, using an emergency laboratory, a very unsuitable hothouse, and a staff which was not yet sufficiently trained. In addition, most of the soils concerned were very difficult to handle, being exceptionally wet and sticky. Notwithstanding those very unfavourable conditions, nearly 200 samples could be analysed during one winter-season (from November to April) by laboratory personnel comprising one senior technical assistent (chief of the laboratory), two junior technical assistents and two laboratory helpers. It will undoubtedly be possible to increase the productivity considerably, using better equipment and trained personnel, and a still more important improvement will be reached if the macro-analyses can be replaced by micro-analyses, consequently reducing the work of sampling and transportation of the samples, the size of the hothouse and the incubator, and the amount of work at the laboratory.

V. *Discussion.* The conclusion seems to be justified that we now have a method that enables us to determine the rate of mineralisation of nitrogen in humus, with sufficient accuracy, much quicker and easier than was possible using only field-trials and the accumulated experience of farmers. It is true that the method described has more than one shortcoming, and only well-trained and critical investigators can get reliable results using the method; nevertheless it enables us to determine analytically the nitrogen requirement of soils. Until now only the requirements of K and P could be determined by laboratory methods.Thus this result is rather encouraging and satisfying, but the purposes for which this method can be used must also be considered, since it must be kept in mind that there is decidedly less need of analytical determination of the nitrogen requirement in soils, than that of K and P. The effect of nitrogen on the plants is much more visible than the effect of K or P. Therefore an experienced farmer can estimate the nitrogen he has to apply for a successfull development of the crops much more easily and accurately than the K and P fertilizers. In countries with active and sufficiently intelligent farmers, most of them will be able to estimate the requirement of nitrogen fairly well merely by experience. But in some special cases this laboratory-method can be very useful. Among

other things it will be a welcome air for investigations on the effect of organic manure and on the formation and disintegration of humus. This field of research is becoming more and more important and fundamental for agricultural science, where the main problem is no longer how to increase the yields, but how to maintain them on the present high level without too great an expense. For this research it is necessary to have a reliable method to determine the rate of mineralisation of nitrogenous compounds in humus.

A second application of this method is the investigation and evaluation of uncultivated land, or of land under very extensive and primitive exploitation, when measures must be taken to improve the methods of cultivation. In our country consequently only some tidal marshes, estuaries and shallow coastal waters will offer opportunities for such determinations before starting the enclosure and reclamation of those areas. But in vast and only partly cultivated countries all land for the expansion of the arable area must be selected on the score of a carefull investigation of the qualities of the soils concerned. Among these important qualities the nitrogen requirement occupies one of the most fundamental positions.

In addition to the two cases already mentioned, this method can become very valuable for the investigation of soils under special abnormal conditions, as for instance when soils by any catastrophe suddenly become spoiled, changing entirely the conditions in them. A very spectacular example of such a catastrophe is the flooding of old cultivated land by seawater as a result of inundations during the last war in the western Netherlands and in Flanders. Here this method was used for the first time on a moderately large scale, and it scored a success.

Summary

A new method is described for the determination of the rate of liberation of mineralised nitrogen from the organic form in humus. It is emphasized that only through this determination can a correct indication of the nitrogen-fertilisation requirements of the soils be obtained, since it is not the mineralised nitrogen available at a certain moment in the soil, that indicates the nitrogen supply for nutrition of plants but the amount of nitrogen that can be produced per unit of time by microbes from the large stock of nitrogen in the humus of the soil. Under natural conditions it is impossible to accumulate so much mineral nitrogen as to make this stock cover the total requirement of any crop during a whole season. Therefore the plants

depend for their nutrition during part of their growth on the continuous mineralisation of humus.

The method described is based on the consideration that the measurement of the rate of mineralisation is only reliable if it is performed during a period of very low concentration of mineral nitrogen in the soil. Therefore all mineral nitrogen must be extracted from the soil samples before the regeneration of the mineralised nitrogen can be measured. The extraction is done by cultivating a fast-growing crop on the samples and not by percolation with water, to prevent an unfavourable change in the structure of the soil. The regeneration is then measured by a series of analytical determinations of the water-soluble nitrogen in the samples, while these samples are kept in a room under controlled temperature and moisture, both adjusted to approximately the ideal conditions for the mineralisation.

The results of the consecutive analyses are plotted against time. The slope of the curve obtained is a measurement of the rate of liberation of nitrogen.

As far as could be contemplated theoretically and in the light of experience collected during the first serial application of this method, it is criticised and its value is discussed.

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