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THE DECOMPOSITION OF GREEN MANURES IN SOIL[1].

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(With Eleven Text-figures.)

THE decomposition of green manures in soil has been very extensively studied, but most of the work has been done mainly to measure the rate of formation of the end products of decomposition. The evolution of carbon dioxide has been used by many workers as a criterion for the decay of organic matter. The production of ammonia and the ultimate accumulation of nitrates have been utilised by others to measure the rate of decomposition. Neither of these measurements, however, supplies precise information concerning the processes that govern the liberation of plant nutrients in an available form.

When a green manure is turned under, the micro-organisms present in the soil break down the complex plant material into simpler substances, part of which they consume for building their body tissues, leaving the rest as by-products. The decomposition of a plant material is, therefore, controlled by the organisms that exist in the soil and the environmental conditions under which they function on the one hand, and by the type and variety of the chemical complexes, of which the plant is composed, on the other.

In the present paper the decomposition of green manures in soil under laboratory conditions has been followed by determining periodically the changes taking place in the various plant constituents, such as the carbohydrates and allied products (Series I) and the nitrogen transformations (Series II).

SERIES I.

Experimental.

Four substances—young tares, young mustard, sugar-beet tops and mature mustard—were used. All the materials were rapidly dried at 55–60° C. and finely powdered. The light sandy soil employed was ob-

[1] This paper is an abridged form of the thesis approved for the Degree of Doctor of Philosophy in the University of London.

tained from the headland of one of the green manure experimental fields at the Woburn Experimental Station. It was air dried and passed through a 1 mm. sieve. Each material was added to the soil at the rate of 5 per cent. on the air-dry basis, and water was carefully mixed in to bring the water content of the whole to about 15 per cent. The mixture was put into shallow Petri dishes and incubated at air temperature in a room that was not subject to great fluctuations of temperature. The dishes were kept loosely covered with other Petri dishes of the same size to exclude dust and to reduce evaporation, while allowing free access of air. The loss of moisture was made up very frequently and the samples were aerated by stirring. Separate samples were incubated for the different stages of decomposition. Five sets of soil samples were laid out in this way, one for each of the four green manures and the fifth for the soil alone, which served as a control.

The composition of the green manures as analysed by the methods outlined below is given in Table I. The same determinations, with the exception of lignin and ether extract, were made on the decomposing mixtures.

(1) *Total organic matter.* This was estimated by the loss of weight on ignition.

(2) *Water-soluble organic matter.* An aliquot quantity of the filtrate, representing 1 gm. of the material, obtained by shaking with 100 parts of water for 1 hour, was evaporated to dryness and the loss of weight on ignition was taken as water-soluble organic matter. In the case of soil, 25 gm. were shaken with 125 c.c. water and 50 c.c. taken for evaporation. The ash obtained from this determination was taken as soluble mineral matter.

(3) *Ether extract.* 1 gm. of the substance was extracted with ether in a Soxhlet extractor; the extract was evaporated and the residue weighed.

(4) *Total furfuraldehyde.* This was determined by the usual method of distillation with 12 per cent. HCl and precipitation with phloro-glucinol. 20 gm. of the soil were used for the determination.

(5) *Cellulose.* Cellulose was determined by the method described elsewhere[2].

(6) *Lignin.* Lignin was determined by the Schwalbe method as described by Norman[4].

(7) *Nitrogen.* By the Kjeldahl method.

(8) *Soil reaction.* pH values were determined by the quinhydrone method.

Table I. *Chemical composition of the green manures and of soil,*
expressed on 100 *gm. dry matter.*

	Young tares	Young mustard	Sugar-beet tops	Mature mustard	Soil
Total organic matter	66·60	73·62	81·57	90·81	2·920
Water-soluble organic matter	11·65	22·40	40·20	19·40	0·025
Water-soluble mineral matter	5·35	8·00	7·00	3·70	0·016
Ether extract	2·24	2·43	1·90	1·14	—
Total furfuraldehyde	5·45	6·40	6·65	12·10	0·089
Cellulose	9·71	9·01	7·39	23·32	0·060
Lignin	12·57	13·76	7·38	20·90	—
Nitrogen	3·70	3·65	2·74	1·52	0·114
Potash (K_2O)	2·90	3·85	5·98	—	—

Results.

The decomposition undergone by the various constituents of the green manures is shown in Figs. 1–4. The figures represent the percentage loss of each constituent. They were obtained by subtracting the amount of each of the constituents present in the control soil at the different periods of incubation from those present in the manured soils calculated back to the original material. The percentage loss was worked out therefrom.

Discussion.

There is a very rapid fall of soluble organic substances during the first 4–8 days in the case of all the four plant materials. The rate then falls off and there is very little decomposition after 25 days. Mature mustard contains almost as much soluble organic material as young mustard, yet it loses appreciably more, especially during the first week. The rapidity of decomposition of hemicelluloses as judged by the loss of total furfuraldehyde is about the same in the case of young tares, young mustard and sugar-beet tops. Mature mustard, however, decomposes more slowly though it contains a greater abundance of this group of substances. This is because it contains less soluble nitrogenous compounds than the other three (Series II).

There is no appreciable loss of cellulose during the first 4 days, whereas a considerable amount of the soluble organic matter and hemicelluloses decomposed during that time. The easy and rapid availability of these two latter classes of compounds to the micro-organisms during the opening stages of decomposition is thus evident. A great loss of cellulose takes place during the next 4 days in the case of young tares, young mustard and sugar-beet tops. The rate of disappearance of cellulose in mature mustard is slower on the whole. The comparatively slow
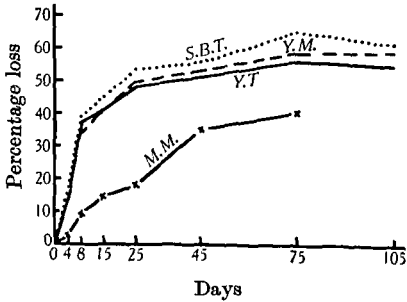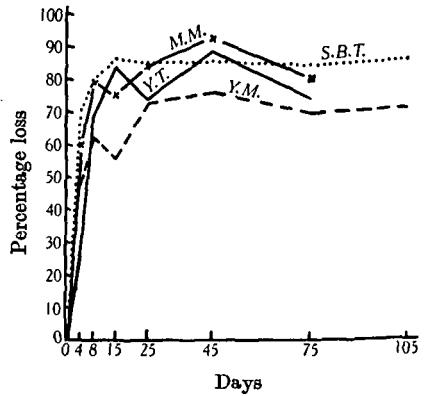
Fig. 1. Decomposition of total organic matter.

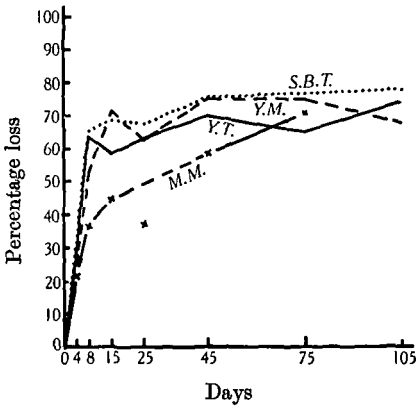Fig. 2. Decomposition of soluble organic matter.
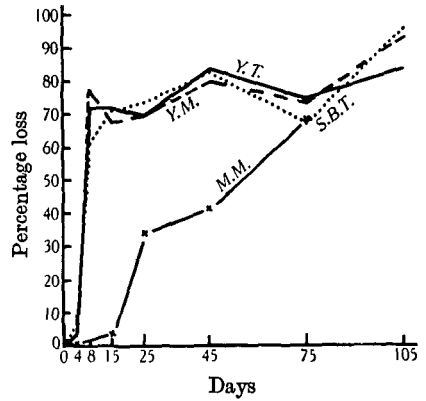
Fig. 3. Decomposition of total furfuraldehyde.
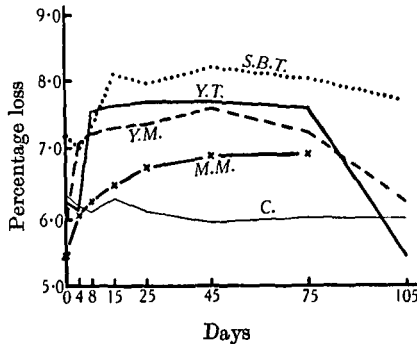
Fig. 4. Decomposition of cellulose.

Fig. 5. Soil reaction.

S.B.T. Sugar-beet tops.　*Y.M.* Young mustard.　*Y.T.* Young tares.
M.M. Mature mustard.　*C.* Control.

decomposition of cellulose in mature mustard is principally due to the high content of lignin which, by virtue of its encrustation on the cell walls, acts as a barrier to the utilisation of cellulose by micro-organisms. But, in view of the fact that the proportion of lignin to cellulose is much higher in the case of young tares, young mustard and sugar-beet tops than in mature mustard, it seems that the rapid availability of cellulose in the young plant materials is only apparent. It is because of the low cellulose content of these three plant materials as compared with their available nitrogen contents that almost the whole of that cellulose is required and used up by the micro-organisms. In other words the amount of available nitrogen in these three plant materials is more than sufficient to balance the total available carbohydrate, viz. the sugars, the hemicelluloses and cellulose. The reverse is true in the case of mature mustard, so that, after the first rapid removal of the soluble carbohydrates together with the soluble nitrogenous compounds, the other carbohydrate compounds decompose slowly as the rest of the nitrogenous substances become available. This is more clearly shown in the data presented in Table II. The sum of the soluble carbohydrates (soluble organic matter − total soluble N × 6·25), hemicelluloses (total furfural × 2), and cellulose is taken as the total available energy material and the sum of the total water-soluble nitrogen plus the nitrogen hydrolysed by H_2O_2 (Series II) is taken as the total available nitrogen. It has been shown that for each part of nitrogen assimilated, fifty to sixty parts of cellulose [12] and twenty to forty parts of hemicelluloses [13] are decomposed. Taking an average of these figures, the energy material that would be required for the total amount of available nitrogen present in each plant material is calculated and shown in the last column of the table.

Table II.

	Total energy material present	Total available nitrogen present	Energy material equivalent to available nitrogen (calculated)
Young tares	26·1	2·47	105
Young mustard	37·6	2·13	90
Sugar-beet tops	54·5	1·74	73
Mature mustard	63·7	0·81	34

Young tares, young mustard and sugar-beet tops contain relatively little carbohydrate in proportion to the available nitrogenous compounds present. Hence a very rapid and immediate decomposition takes place.

The total organic matter disappeared less slowly in the case of all

2–2

the four plant materials than the hemicelluloses, cellulose and soluble organic matter. This is primarily due to the accumulation of the more resistant constituents, chiefly lignin, which does not decompose as readily as the other plant constituents. Lignin was not determined in the decomposing materials, mainly because there is no suitable method for estimating it in a mixture of soil and plant material. The formation of new organic matter in the tissues of the fungal mycelium and bacteria is also a cause of the less rapid decomposition of total organic matter.

These results are in keeping with the findings of Rege[7], Norman[5], Waksman and his associates[13, 14, 15] and others cited by Tenney and Waksman[11]. Rege first observed that in mature plant materials pentosans form the principal food material for the micro-organisms. This was later confirmed by Norman, who emphasised the importance of cellulose along with the pentosans as the chief source of energy material for the organisms. He further supported the view of Rege that lignin acts as an inhibitory agent. The work of Waksman and his colleagues showed that the decomposition depends on the nature and age of the plant material. The younger the plant the more rapidly does it decompose. The decomposition is influenced by the type of compounds present in the plant material, some like sugars, hemicelluloses and cellulose being more easily available than the others, *e.g.* lignin, resin and waxes. In confirming the previous work of Hutchinson and Richards[3] they further showed that there is a definite proportion between the amounts of available carbohydrate and available nitrogen for the requirements of the activities of the micro-organisms. Hence plants having a balanced proportion of these two classes of compounds decomposed more rapidly than others.

The nitrogen figures are more fully discussed in the second series of experiments.

The effect of green manures on the soil reaction is well marked (Fig. 5). After a small increase of acidity during the initial stages of decomposition the soil tends to become alkaline and remains so for a major part of the period of incubation. The increase in acidity and then in alkalinity seems to correspond with the production of organic acids from sugars and starches followed by, or simultaneously with, the production of ammonia. The fall in pH values during the later stages of decomposition corresponds to the formation of nitrates (Series II). Soil mixed with sugar-beet tops, however, remains alkaline even after nitrification has set in. This is due to the large amount of potash in the sugar-beet tops, which tends to keep the soil alkaline. Other differences in

the soil reaction due to the various green manures can also be similarly explained. The reaction of the soil is controlled by two factors: (1) the products of decomposition, viz. the organic acids, ammonia and nitrates, and (2) the liberation of the ash constituents of the green manure. The preponderance of one over the other decides the condition of the soil at any given time. The work of White[16], Stephenson[9, 10] and Smith and Humfield[8] goes to show that the soil becomes alkaline during the early stages of decomposition but becomes acidic later on.

SERIES II.

The second series of experiments was designed to determine the nitrogen changes that take place during the decomposition of the green manures under study. Besides measuring the accumulation of ammonia and nitrates an attempt was made to observe the changes taking place in the potentially available class of compounds other than those soluble in water.

Mixtures of soil plus green manures were laid out in Petri dishes in the same way as in Series I, and samples were taken for analysis at the same intervals but for 65 days only.

The following determinations were carried out:

(1) *Total nitrogen*. Total nitrogen was determined by the salicylic-thiosulphate method to include nitrate nitrogen[1].

(2) *Water-soluble ammonia nitrogen*. A suitable quantity of the soil was shaken with five times its weight of water for 1 hour; an aliquot volume of the clear filtrate representing 20 gm. of the soil was distilled with magnesia and ammonia collected in standard acid.

(3) *Nitrate nitrogen*. Nitrate nitrogen was determined from the same filtrate as the ammonia, the contents of the flask being made up with water and 2–3 gm. of Devarda alloy added.

(4) *Total water-soluble nitrogen*. On another aliquot from the soil extract total soluble nitrogen was determined according to Ranker[6]. Total soluble nitrogen minus ammonia plus nitrate nitrogen is taken as water-soluble protein nitrogen.

(5) *Hydrogen peroxide nitrogen*. The action of dilute H_2O_2 was used to determine the potentially available class of nitrogenous compounds. The residue after water extraction was treated with 60 c.c. of a 3 per cent. solution of hydrogen peroxide. After the initial frothing had ceased, it was boiled for 15 min. The extract was filtered through a Buchner funnel, the residue washed and the filtrate made up to a definite volume. An aliquot portion of the filtrate was distilled with magnesia

and another with caustic soda. Each time ammonia was collected in separate quantities of standard acid. The residual soil was then further treated with another 60 c.c. of 3 per cent. hydrogen peroxide and ammonia determined as in the previous case. A third extraction was then made on the residual material and ammonia determined again as before. Thus each sample gave two forms of nitrogen three times in succession.

Assuming that the oxidising power of 3 per cent. hydrogen peroxide, under standard conditions of time and volume, measures the availability of the less easily available class of compounds, this group is sub-divided into two classes: (1) that which would be more easily available; this is measured by the distillation of the extract with magnesia and called "H_2O_2 nitrogen I," and (2) the rest of the nitrogen, which is measured by the difference between the nitrogen evolved by the caustic soda distillation and that by the magnesia distillation. This form of nitrogen is referred to as "H_2O_2 nitrogen II." The nitrogen evolved by the caustic soda distillation is referred to as the "Total H_2O_2 nitrogen."

The green manures were subjected to this system of analysis, the results of which are given in Table III.

Table III. *Different forms of nitrogen in the green manures, expressed on* 100 *gm. dry matter.*

			Young tares	Young mustard	Sugar-beet tops	Mature mustard
1.	Total nitrogen		3·70	3·65	2·74	1·52
2.	Water-soluble nitrogen					
	Ammonia nitrogen		0·096	0·249	0·089	0·049
	Nitrate nitrogen		0·096	0·121	0·160	0·034
	Total soluble nitrogen		1·18	1·43	1·27	0·60
	Percentage of total nitrogen		31·90	39·20	46·30	39·50
3.	H_2O_2 nitrogen I	1st extraction	0·460	0·114	0·124	0·029
		2nd extraction	0·226	0·179	0·049	0·032
		3rd extraction	0·051	0·111	0·041	0·019
4.	H_2O_2 nitrogen II	1st extraction	0·383	0·102	0·162	0·056
		2nd extraction	0·169	0·133	0·041	0·047
		3rd extraction	0·002	0·064	0·052	0·031
5.	Total available nitrogen $(2+3+4)$		2·47	2·13	1·74	0·81

It is interesting to note that mature mustard contains relatively as much nitrogen in a soluble form as young mustard. It is the nitrogen soluble in water which, according to Whiting and Richmond[17], is responsible for the early nitrification of plant materials even though they may contain less total nitrogen. Young tares contain considerably more nitrogen oxidised by H_2O_2 both absolutely as well as relatively to

the total nitrogen, than the other three plant materials. Looking to the nitrogen oxidised at each of the extractions it will be noticed that young tares and sugar-beet tops liberate a major portion of the nitrogen at the first extraction both as H_2O_2 nitrogen I as well as H_2O_2 nitrogen II. In the case of young as well as mature mustard, however, it is a more gradual liberation.

Results and Discussion.

The results of the decomposition studies of the four green manures are presented in Figs. 6–11. As the nitrogen liberated by hydrogen peroxide at the three different extractions did not show any noticeable differences, the total of the three extractions is given together. All the results have been calculated on the original dry matter and represent nitrogen present in the soil mixtures at different periods.

The loss of total nitrogen, especially in the case of young tares, young mustard and sugar-beet tops is considerable. A similar loss of nitrogen was noticed in the first series of experiments. It was then not clear whether that loss was actual or only apparent as the nitrogen determinations did not include nitrates. In the present series of experiments, where the total nitrogen was determined so as to include nitrates, the loss becomes actual. It has been shown previously (Table II) that these three plant materials have a very high proportion of total available nitrogen to total energy material. In other words they have a narrow nitrogen-carbon ratio. This accounts for the loss of nitrogen noticed in these experiments.

Similar losses of nitrogen have been observed by other workers. Tenney and Waksman [11] noticed a decrease of total protein during the decomposition of alfalfa which contained 2·58 per cent. total nitrogen. But the more striking experiments are those of Zolcinski and Musierowicz [18, 19] who, while studying the decomposition of lucerne and red clover, noticed large losses of total nitrogen. These are materials both very rich in nitrogen. The present experiments are, therefore, in accord with the results obtained by them.

The opening stage of the decomposition is characterised by a rapid loss of soluble nitrogen during the first 4 days (Fig. 7). During the same time there is an increase in the amount of total H_2O_2 nitrogen (Fig. 10). It seems therefore that the soluble nitrogen is being converted into more complex nitrogenous compounds, very probably the synthesised protein matter of the fungi that are growing very vigorously at that time. A part of the increase in total H_2O_2 nitrogen may also be due to the
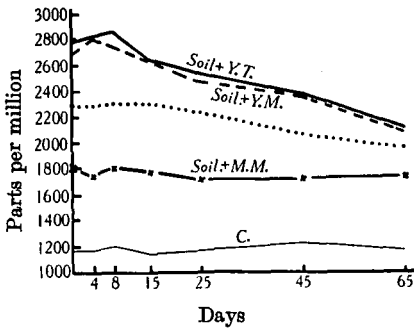
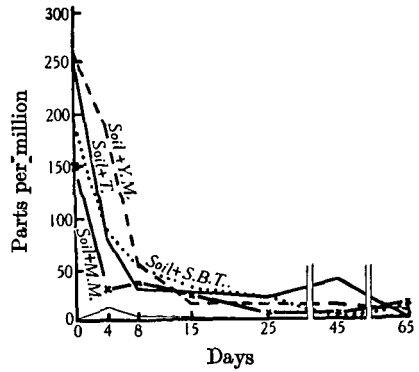Fig. 6. Changes in total nitrogen.

Fig. 7. Changes in water-soluble protein nitrogen.
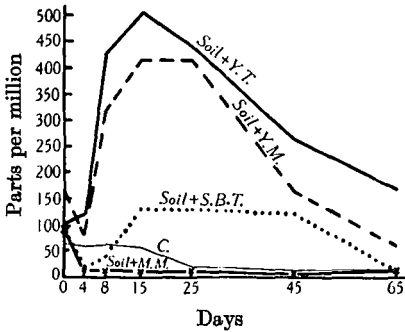

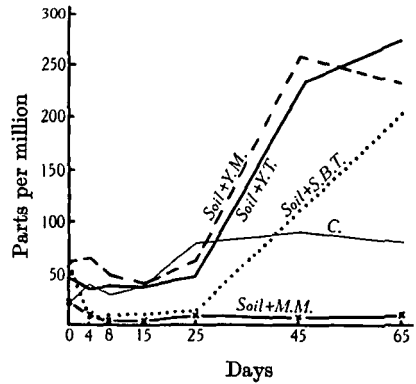Fig. 8. Changes in water-soluble ammoniacal nitrogen.
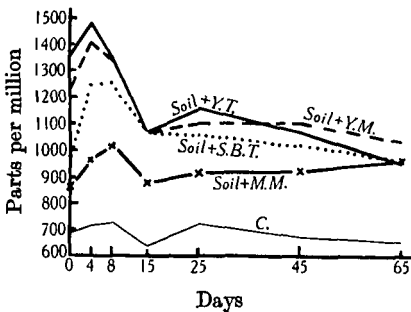

Fig. 9. Changes in nitrate nitrogen.
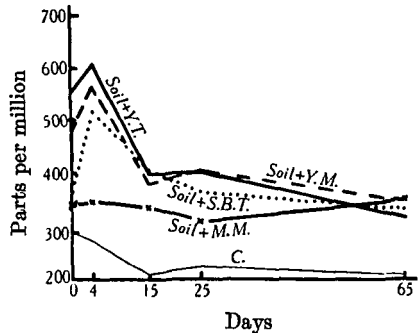

Fig. 10. Changes in total hydrogen peroxide nitrogen.

Fig. 11. Changes in hydrogen peroxide nitrogen II.

$Soil + Y.T.$ Soil + young tares. $Soil + Y.M.$ Soil + young mustard.
$Soil + S.B.T.$ Soil + sugar-beet tops. $Soil + M.M.$ Soil + mature mustard.
$C.$ Control.

breaking up of the more complex nitrogen compounds. After 4 days this class of compounds begins to be broken up again, giving rise at the same time to ammonia. A little later nitrification sets in and nitrates begin to accumulate. The late nitrification is mainly due to the large accumulation of ammonia which checks the activities of nitrifying organisms for a time. With the increase of nitrates there is a sharp fall of ammonia and this goes on to the end of the period of incubation. At the same time the fall of the nitrogen oxidisable by hydrogen peroxide continues though more gradually than before. The water-soluble protein nitrogen remains almost constant at a very low level after the first rapid consumption (Fig. 8).

Mature mustard differs considerably in its chemical composition from the other three plant materials. Hence the nature of changes taking place in its nitrogen is strikingly different. It does not lose any appreciable amount of total nitrogen. There is a small but definite increase in the amount of total H_2O_2 nitrogen after the first 15 days, unlike the other green manures. This increase in nitrogen oxidisable by hydrogen peroxide is very probably due to the activities of the micro-organisms breaking down the hemicelluloses and cellulose which are present in larger quantities in this plant material than in the other three. The lack of available nitrogen is shown by the depression caused in the level of ammonia and nitrates below that of the control soil. In other words the micro-organisms are utilising the soluble nitrogen present in the soil for the decomposition of the soluble carbohydrates and the hemicelluloses. Hence there is no accumulation of ammonia nor of nitrates over a period of 65 days.

To sum up, the results presented in this series of experiments show that when a green manure rich in nitrogen is incorporated with the soil, large losses of nitrogen take place. The loss of nitrogen depends not only on the amount of total nitrogen contained in the green manure but also, and more especially, on the amount of nitrogen that is easily available. The greater the amount of total and available nitrogen the greater is the loss of nitrogen likely to occur. The results also give an insight into the nature of the changes taking place in the nitrogenous compounds of the green manures. The soluble nitrogenous compounds are attacked first and are metabolised into a more complex form of protein. This protein together with that existing in the green manure is then attacked and converted first into the less complex forms (e.g. water-soluble) and subsequently or simultaneously into ammonia and nitrates. Since a green manure rich in nitrogen is almost always comparatively poor in

carbohydrate compounds, there is the risk of a part of the ammonia being lost.

SUMMARY.

Four plant materials of widely different origin and age were used as green manures mixed with soil for decomposition studies under laboratory conditions.

Provided the conditions of temperature, moisture, aeration, and micro-flora, are optimal, the decomposition depends upon the chemical constituents of the plant materials. It is shown that the soluble carbohydrates, hemicelluloses and cellulose are the compounds mainly responsible for the loss of total organic matter during decomposition.

Plant materials containing a balanced proportion of available carbohydrate compounds to available nitrogenous compounds decompose rapidly. Those containing excess of nitrogenous compounds decompose more rapidly and those containing excess of carbohydrate compounds decompose less rapidly. This is true in all cases whether the plant material is a legume or a non-legume. Young plant materials by virtue of their abundance of available nitrogenous compounds decompose more quickly than mature tissues.

When comparatively young plant materials are used as green manure, there is the danger of a loss of nitrogen, the loss depending upon the amount of total and available nitrogen this contains. Not only do they lose nitrogen but they decompose very rapidly, with the result that nitrates accumulate soon after burial. Unless the succeeding crop is sown sufficiently early to utilise these nitrates, they are likely to be lost through leaching under field conditions.

REFERENCES.

(1) A.O.A.C. *Methods of Analysis* (1921), 2nd ed.

(2) DAJI, J. A. *Biochem. J.* (1932), **26**, 1275.

(3) HUTCHINSON, H. B. and RICHARDS, E. H. *J. Min. Agric.* (1921), **28**, 398.

(4) NORMAN, A. G. *Biochem. J.* (1929), **23**, 1353.

(5) —— *Biochem. J.* (1929), **23**, 1367.

(6) RANKER, E. R. *J. Assoc. Off. Agric. Chem.* (1927), **10**, 230.

(7) REGE, R. D. *Ann. App. Biol.* (1927), **14**, 1.

(8) SMITH, N. R. and HUMFIELD, H. J. *Agric. Res.* (1930), **41**, 97.

(9) STEPHENSON, R. E. *Soil Sci.* (1918), **6**, 413.

(10) —— *Soil Sci.* (1921), **12**, 145.

(11) TENNEY, F. G. and WAKSMAN, S. A. *Soil Sci.* (1929), **28**, 55.

(12) WAKSMAN, S. A. *Principles of Soil Microbiology* (1927). Ballière, Tindall and Cox, London.

(13) WAKSMAN, S. A. and DIEHM, R. A. *Soil Sci.* (1931), **32**, 119.

(14) WAKSMAN, S. A. and TENNEY, F. G. *Soil. Sci.* (1927), **24**, 317.

(15) —— —— *Soil Sci.* (1928), **26**, 155.

(16) WHITE, J. W. *J. Agric. Res.* (1918), **13**, 171.

(17) WHITING, A. L. and RICHMOND, T. E. *Soil Sci.* (1927), **24**, 31.

(18) ZOLCINSKI, J. *Rocz. Nank. Poln. i. Lesnych* (1927), **17**, 349. (*Chem. Abs.* **22**, 3725.)

(19) ZOLCINSKI, J. and MUSIEROWICZ, A. *Rocz. Nank. Poln. i. Lesnych* (1927), **17**, 377. (*Chem. Abs.* **22**, 3725.)