CXCV. THE COMPOSITION OF FORAGE CROPS

II. RYE GRASS (WESTERN WOLTHS). CHANGES IN HERBAGE AND SOIL DURING GROWTH

BY ARTHUR GEOFFREY NORMAN AND
HENRY LORIMER RICHARDSON

*From the Biochemistry Section and Chemistry Department, Rothamsted
Experimental Station, Harpenden, Herts*

(Received 22 July 1937)

PRELIMINARY experiments on the developmental changes in composition of rye grass revealed some interesting features, not the least of which was the presence of a water-soluble fructosan in considerable amount in the young grass [Norman, 1936]. A more detailed investigation involving weekly samples of both soil and herbage throughout the growing season has now been undertaken. In the soil analyses the more mobile forms of nitrogen and carbon were studied, since the changes in total nitrogen and carbon during the growth of a single crop of rye grass are likely to be so small as to lie within the experimental error of the determinations. The ammonia and nitrate contents of the fresh soil were measured, and also the ammonia, nitrate and carbon dioxide produced when the soil samples were incubated under standard conditions. Methods of this type have been extensively used here to study the influence of cropping on the soil organic matter [Orchard, 1933; Richardson, 1935; Marshall, 1936]. The ammonia and nitrate in the fresh soil show the amount of nitrogen immediately available to the crop, while the incubation values give an index to the amount of readily decomposable nitrogen and carbon in the soil organic matter.

Methods employed for the grass

The samples of grass were taken from Hoosfield, Rothamsted, from an area under barley in the previous season. The selected area was laid out with 98 plots each $1\frac{1}{2}$ yd. sq. As before, an area of 1 sq. yd. was cut from the plot at the time of sampling. Each sample consisted of 6 sq. yd. taken at random, and bulked. Owing to the variation in the density of the herbage this was insufficient to give reliable figures for increase in dry weight. The bulked samples were treated as in the previous season.

Soil sampling was commenced on 25 April 1936, $2\frac{1}{2}$ weeks before the first grass sample was taken. On 4 May a light dressing of sulphate of ammonia was applied at the rate of $1\frac{1}{2}$ cwt. per acre. The stand of grass was rather thin, and the grass tended to be of a stemmy character. The season was almost uniformly wet, a circumstance which favoured the later growth of clover on the plots which had been cut earlier in the season. A sample was cut and left for hay at the time when the grass was judged by the farm manager to be ripe for this purpose.

The analytical methods employed for the grass were as described in the previous paper [Norman, 1936].

A. COMPOSITION OF RYE GRASS

The approximate rate of growth of the rye grass may be seen from Table I. These figures do not fall on a smooth growth curve because insufficient plots

Table I. *Yield of rye grass per sq. yd. (first cut)*

Sample	Date	Weight oven-dry g.	Sample	Date	Weight oven-dry g.
1	12 May	23	8	30 June	161
2	19 "	54	9	7 July	161
3	26 "	64	10	14 "	165
4	2 June	93	11	20 "	151
5	9 "	126	12	26 "	126
6	16 "	166	13	21 Sept.	—
7	22 "	150			

were cut to even out the irregularity in the density of the grass, but they leave little doubt that there was no increase in dry weight per unit area after mid-June.

Non-structural constituents (Table II)

The changes observed in ash and crude protein present no special features, and are of the type usually observed in a growing crop. The anomalous figure for ash in sample 4 was due to the presence of some clay through sampling under

Table II. *Some non-structural constituents of rye grass*

All analyses expressed as % on oven-dried material

Sample	Ash	Crude protein	Cold water- soluble	Protein in aqueous extract	Fructose before hydrolysis	Fructose after hydrolysis
1	9.8	11.7	49.5	3.3	2.1	25.6
2	8.5	9.3	52.5	4.4	3.1	26.9
3	8.1	9.1	56.9	4.7	3.6	30.1
4	(10.3)	7.7	46.5	4.1	1.8	28.1
5	5.5	6.9	44.4	2.6	3.2	26.8
6	5.5	6.4	39.0	1.9	2.1	21.3
7	6.9	6.0	31.5	2.0	2.4	17.0
*Hay	6.4	5.6	26.4	2.9	5.1	11.8
8	7.0	5.4	30.7	2.2	2.3	14.6
9	6.6	4.7	25.1	1.6	2.0	12.6
10	6.8	4.7	20.7	1.5	1.0	7.0
11	7.5	4.2	15.7	1.2	1.0	5.7
12	7.9	5.1	15.7	1.6	0.9	3.2
13	5.4	6.7	9.9	2.1	0.1	0.4
†s.e. of mean ±	0.10	0.09	0.23	0.07	0.09	0.17

* Cut with Sample 7; left 1 week in field.

† The standard errors of these determinations are calculated on all samples, and include those in Table V.

bad weather conditions. As observed previously there is a high percentage of water-soluble material in this grass in its younger stages, only a small portion of which is nitrogenous. The major part is fructosan, which constituent rises to a peak towards the end of May subsequently falling sharply as the grass matures and becomes senescent. The free sugar consists largely of fructose, but no particular significance is to be attached to the amounts found, since this is believed to be in part a result of the drying procedure. In Sample 3, when

dried, 3.6% free fructose was found. Grass cut at the same time and extracted when fresh with hot 95% alcohol contained only 1.64% total reducing sugar, expressed as fructose, of which 1.57% was fructose. The peak content of fructosan occurred while the dry weight was still rapidly increasing, so that this polysaccharide must be of the nature of a temporary reserve. The peak was appreciably lower than that recorded in the previous season and coincided approximately with the time of full emergence of the head.

The distribution of fructosan within the plant at the time of the fourth sampling was studied (Table III). It appears that the stem is the chief place

Table III. *Distribution of fructosan in rye grass. Sample 4.*
Cut 2 June

Region	Proportion of aerial parts	% free fructose	% fructose after hydrolysis
Leaves	25.4	1.0	24.4
Stems	51.5	1.9	35.4
Heads	23.2	1.5	17.3
Root	—	0.7	25.0

of storage. Division of the stem into internodes showed that the first internode above ground level is richest in this constituent, 42.8% being found. In the second internode only 34.1% was present.

The fructosan, soluble protein and soluble ash constituents do not together account for all the cold water-soluble material, and evidence of the presence of a glucose polysaccharide, hydrolysed considerably less easily than fructosan, has been obtained. The satisfactory determination of a mixture of these two polysaccharides has not yet been solved, partly because the conditions necessary for complete hydrolysis of the glucosan result in some destruction of fructose. The new polysaccharide is present in considerably smaller amounts than the fructosan. In sample 3, which contained 30.1% fructosan, about 6.7% glucosan was detected.

Structural constituents (Table IV)

The crude fibre determination has been abandoned on the grounds that it is both inaccurate and misleading [Norman, 1935], and reliance is placed instead on the direct determination of cellulose and lignin. The cellulose content increases steadily and rapidly while the dry weight of the plant is increasing and the grass is approaching maturity. The largest percentage increase between the time of the 6th and 7th samples does not in fact represent a spurt in cellulose production, but is partly the result of a fall in other constituents, as may be seen later in Fig. 1 (p. 1560) in which the cellulose present is shown on a basis of g. per sq. yd. After the point at which no increase in dry weight occurs, the percentage of cellulose still rises, though less rapidly, presumably by conversion of some other constituent. There is a limit to this change, for the extremely old grass left till September, Sample 13, is not much richer in cellulose than that cut in late July.

The yield of furfuraldehyde from the cellulose, and therefore the xylan associated with the cellulose, does increase with age, but not in any very steady manner. When the xylan is calculated as % of the cellulose, there is an indication of a tendency to increase during the period of active growth, as was observed in the experiments of the previous year, and then a decrease during senescence. The changes are somewhat irregular as were those found for the barley plant by Phillips & Goss [1935].

Table IV. *Some structural constituents of rye grass*

All analyses expressed as % of oven-dried material

Sample	Cellulose	Furfur- aldehyde from cellulose	Xylan in cellulose	Total furfur- aldehyde	Furfur- aldehyde from poly- uronides	Lignin	N in lignin
1	26.1	4.3	6.7	7.4	3.1	3.6	0.3
2	26.4	4.5	7.0	8.8	4.3	4.7	0.3
3	28.9	5.4	8.4	9.1	3.7	5.2	0.3
4	32.0	5.5	8.6	9.8	4.3	5.9	0.3
5	32.2	6.0	9.3	10.9	4.9	7.0	0.3
6	33.8	5.9	9.2	11.4	5.5	8.7	0.3
7	40.4	6.1	9.5	12.4	6.3	9.0	0.3
Hay	43.2	6.4	10.0	13.3	6.9	10.4	0.2
8	38.3	6.3	9.8	12.6	6.3	9.1	0.3
9	39.9	6.3	9.8	13.4	7.1	9.6	0.3
10	43.2	6.2	9.7	13.8	7.6	10.5	0.3
11	47.1	6.0	9.4	14.3	8.3	11.2	0.2
12	46.2	6.3	9.8	14.1	7.9	11.1	0.3
13	48.8	6.4	10.0	15.1	8.6	16.4	0.3

*S.E. of
mean $\pm$

* The standard errors of these determinations are calculated on all samples and include those in Table VI.

No satisfactory direct method for the evaluation of the polyuronide hemicelluloses has yet been devised, and accordingly the difference between total furfuraldehyde yield and that from the xylan in cellulose is taken as a measure of this constituent. The changes appear to be similar to those exhibited by the cellulose, namely a rapid increase during the period of active growth and subsequently a more gradual increase during the senescent period.

The presence of lignin in forage crops is believed to affect digestibility adversely, and the "running off" of grass is always said to be a result of lignification. The results obtained indicate that the deposition of lignin is a gradual process and not a change which occurs especially at maturity. The figures slightly over-estimate the lignin present, because of the interference produced by the presence of protein, as a result of which the lignin residue contains some nitrogen. No satisfactory correction is applicable nor can the disturbance be wholly overcome. It will be seen that the nitrogen retained by the lignin was almost uniform, and presumably therefore the error is nearly constant. The extremely old grass sample taken in September was considerably higher in lignin than any of the mature samples. Lignin formation seems to continue when other activities have practically ceased.

Taken together, the main structural constituents, cellulose, hemicelluloses, and lignin increase progressively with age. If a rough estimate of the hemicellulose content is made by taking twice the furfuraldehyde figure, it may be said that in the youngest sample these structural constituents account for not less than 36 % of the dry weight; in Sample 7, at maturity, not less than 62 %; and in Sample 11, late in July, not less than 75 %.

Changes in haymaking

The method of sampling did not permit of a satisfactory estimate of the loss occurring during haymaking, but a comparison of the contents of cellulose and lignin in the hay and the appropriate sample cut at the same time suggests that the loss was about 10 %. This seems to be due to removal of some ash,

protein and fructosan. At the same time the proportion of nitrogen soluble in cold water increased, as did also the free fructose, presumably by enzymic hydrolysis of the fructosan.

Some physiological aspects

The presence of the polysaccharide fructosan as a major constituent of young grass, and its disappearance on maturity, raises certain physiological questions which cannot yet be fully answered. Unlike the cereals, which also contain fructosan in the young plant, rye grass does not store any very large amount of carbohydrate in the seed, and instead of being utilized by transformation into starch, as is conceivably the case in cereals, the fructosan in the grass is presumably utilized for general development. There is a strong indication that the structural constituents and, in particular, cellulose, which continue to increase after the dry weight of the plant has reached its maximum, do so at the expense of the stored fructosan. This may be seen from Fig. 1 in which are plotted the

Fig. 1. Cellulose, fructosan and lignin contents of rye grass expressed on an equal area basis.

●—● Cellulose. ■—■ Fructosan. — Lignin.

cellulose, lignin and fructosan in the grass from 1 sq. yd. The approximate point at which the growth curve reached its maximum, after which there was no increase in dry weight, is indicated, and it will be seen that the subsequent fall in fructosan is in part balanced by a rise in cellulose. Since there are no direct figures for hemicelluloses these cannot be plotted, but nevertheless the indication from furfuraldehyde yield is that they too increased concurrently.

Fig. 1 shows also that on an area basis the fructosan maximum occurs later than the peak in percentage content owing to the increase in dry weight. The amount present at the time of taking Sample 6 was nearly $3\frac{1}{2}$ cwt. per acre.

Various factors connected with the formation and utilization of fructosan remain to be elucidated, including seasonal and manurial effects.

Composition of second growth

Certain of the sets of plots were recut later in the season in order to obtain information as to the composition of second growth of the herbage. As a result of the wet summer, clover and weeds in some cases provided serious competition

to the new growth. These experiments are only of a preliminary nature as the series is incomplete, and the samples represent material cut at increasing intervals from sets of plots themselves not originally cut at the same date. The analyses for non-structural constituents are given in Table V. The crude protein

Table V. *Some non-structural constituents of second growth rye grass*

All analyses expressed as % of oven-dried material								
Sample	Date cut	Weeks after first cut	Ash	Crude protein	Cold water-soluble	Protein in aqueous extract	Fructose before hydrolysis	Fructose after hydrolysis
1-1	15 June	5	9.2	10.5	33.0	3.4	2.2	14.2
2-1	29 "	6	9.0	7.5	29.7	2.6	2.5	12.2
3-1	14 July	7	7.4	7.1	27.4	2.3	1.7	8.9
4-1	27 "	8	7.5	5.4	20.3	1.6	1.3	5.5
5-1	11 Aug.	9	8.8	6.7	18.0	1.9	1.2	6.1
7-1	11 "	7	8.2	8.0	26.9	2.2	1.8	4.6
6-1	18 "	9	8.9	7.8	21.3	2.0	1.1	5.1
7-2	25 "	9	8.4	8.2	24.4	3.1	1.6	6.5

content was inclined to be higher than on first growth grass of approximately the same stage of development. The most interesting feature is the low percentage of cold water-soluble material and of fructosan, relative to the protein content. In first growth grass with a crude protein figure of 7.7 % (Sample 4), over 46 % was water-soluble, out of which 28 % could be accounted for by fructosan. In the second cuts of approximately equal or higher protein contents, these figures are not approached. The comparison of Sample 3-1 with 7-1, and Samples 5-1 and 6-1 with 7-2, groups respectively of equal age, shows that considerable differences in composition may result according to the period over which the growth takes place. This is not surprising since equal age does not necessarily imply an equal stage of development, which may be affected by many factors.

A low percentage of water-soluble constituents implies more of the structural cell wall substances, as is observed (Table VI). Cellulose, lignin and polyuronide hemicelluloses are all higher on these samples than on first growth of approximately similar protein content. For example, Samples 4 and 2-1 are almost equal in crude protein, but the sum of cellulose + lignin in the former amounts to 37.9 % and in the latter to 48.0 %. Similarly with Samples 5 and 5-1, the totals are 39.2 and 52.2 %.

Table VI. *Some structural constituents of second growth rye grass*

All analyses expressed as % of oven-dried material							
Sample	Cellulose	Furfur-aldehyde from cellulose	Xylan in cellulose	Total furfur-aldehyde	Furfur-aldehyde from polyuronides	Lignin	N in lignin
1-1	38.4	6.0	9.4	12.2	6.2	8.2	0.4
2-1	37.9	5.9	9.2	12.0	6.1	10.1	0.4
3-1	33.2	5.7	8.9	11.5	5.8	9.2	0.3
4-1	42.9	5.8	9.0	13.3	7.5	11.5	0.2
5-1	40.6	6.0	9.4	12.9	6.9	11.6	0.3
7-1	36.4	6.0	9.4	11.5	5.5	10.0	0.3
6-1	38.4	5.8	9.0	12.1	6.3	11.0	0.3
7-2	39.2	5.7	8.9	12.3	6.6	10.4	0.4

Because of the incompleteness of the series it is impossible to generalize from these results, but they give a strong indication that second growth grass is

more "fibrous" than the first growth; that is to say the amount of structural material present is higher, and the amount of water-soluble constituents, such as fructosan, considerably lower, when considered on the basis of equal protein contents, as is the case when feeding value is in question. If this is applicable to grasses in general it may be the explanation of the belief held by many farmers that autumn grass is not so satisfactory as spring grass even though the nitrogen percentage may be the same. From the analyses obtained the carbohydrates of the second growth would be likely to be less available than those of the first growth.

B. CHANGES IN THE SOIL DURING THE GROWTH OF RYE GRASS

The soil was the heavy clay-loam with flints of Rothamsted; small lumps of chalk were present, and the reaction was pH 8.0. Because the results obtained in studies of the mobile forms of nitrogen and carbon are highly susceptible to changes in the working conditions, the methods employed in the present investigation are described rather fully.

Sampling and analysis

The depth of sampling was 20 cm., a half-cylindrical sampling tool taking a core of 60–70 g. being used. It was pushed in beyond the depth required, and the bottom few cm. of soil discarded. Four samples, each consisting of 6 or 7 cores, were collected in stoppered bottles on each date of sampling. At first the samples were taken entirely at random; later, when the regular sampling of the herbage began, two of the samples were taken from the six plots whose herbage had just been cut, and the other two samples from the plots cut a week earlier. Each of the four samples was analysed or incubated separately, so that the standard error of the determinations gives a combined measure of errors of sampling and analysis.

In the laboratory the soils were broken up by hand, roots and flint fragments being removed, and after thorough mixing the soils were subsampled by quartering. 100 g. were returned to each bottle for the preparation of an extract, 100 g. were transferred to flasks for incubation, 50 g. were used for dry matter determination (24 hr. in electric oven at 105°) and the remainder was air-dried, ground and sieved.

The extracts for ammonia and nitrate determination were made by Olsen's method [1929], slightly modified. 100 ml. of 2 *M* KCl solution were added to 100 g. soil, then enough 2 *N* HCl to give the extract a pH of about 1.0 (to thymol blue), and water to make the total volume of liquid added 200 ml. A few drops of toluene were added as a preservative, and the whole was shaken in a reciprocating machine for 1 hr. The suspension was filtered through a fluted filter (24 cm. diam.), the whole being poured in at once, and the first 25 ml. of filtrate discarded. This stage was always reached on the day of sampling: the filtered extract could be kept several days if necessary in a stoppered flask in a refrigerator.

Ammonia was determined by distilling 100 ml. of the extract, to which 200 ml. of water had been added, from *ca.* 3 g. of freshly ignited MgO; the distillate was collected in *N*/50 acid and titrated to an end-point of pH 4.8 with bromocresol green as indicator.

Nitrate was determined in the same solution by further distillation after adding *ca.* 2.5 g. of powdered Devarda's alloy, and water to replace that lost in the ammonia distillation. Distillation was slow because of frothing, and this gave sufficient time for reduction of the nitrate.

Blanks were determined on all reagents, and the results of the determinations, after blank correction, were calculated on the basis of oven-dry soil.

Ammonia and nitrate in the fresh soil

The soil was first sampled on 25 April, when the grass was still fairly short. It was sampled again on 7 May, 3 days after the application of sulphate of ammonia; then on 11 May; and subsequently at approximately weekly intervals, usually on the dates of cutting the herbage, until 28 July. The results appear in Table VII: for ammonia, the standard error is given at each sampling date because of its variation with time; for nitrate, where the standard error was more constant, a single mean value is given.

Table VII. *Ammonia and nitrate nitrogen in fresh soil*

Sampling date	mg./kg. dry soil														
	Apr. 25	May					June					July			
Ammonia N		7	11	14	19	26	3	9	16	22	30	6	14	20	28
(mean of 4)	1.0	3.7	3.0	4.4	2.2	2.5	3.7	1.9	2.6	1.3	1.1	1.0	1.0	1.2	1.0
s.e. of mean $\pm$	0.06	1.00	0.33	1.46	0.58	0.53	1.07	0.60	1.76	0.06	0.23	0.07	0.07	0.08	0.13
Nitrate N															
(mean of 4)	0.3	0.2	2.3	0.7	0.3	0.1	1.3	1.1	0.2	0.5	0.9	0.5	0.4	0.6	0.5
s.e. of mean								± 0.24							

Since the nitrogen applied as sulphate of ammonia on 4 May was equivalent to approximately 18 mg./kg., the results for soil ammonia show that there was rapid disappearance of most of the added nitrogen. A similarly rapid disappearance in late spring has been recorded on other grassland soils [Richardson, 1932; Eggleton, 1934]. A small amount of extra ammonia was found in the soil until mid-June; this ammonia was unevenly distributed, as may be seen from the high standard errors over this period, and its presence is attributed to the rather thin stand of grass leaving small "pockets" of unabsorbed ammonia in the soil. Very little nitrate was found, the maximum being 2.3 mg. N/kg. a week after adding the sulphate of ammonia. Any nitrate formed would rapidly be taken up by the herbage, but it is probable that much of the added nitrogen was taken up directly as ammonia. The herbage showed a visible response in colour and growth a week after the sulphate of ammonia application, which agrees with the rapid disappearance of ammonia from the soil in suggesting direct uptake without the necessity of nitrification as an intermediate stage [cf. Eggleton, 1935].

The average levels of ammonia and nitrate before adding sulphate of ammonia and after its effect had disappeared were very low, of the order of 1.0 and 0.5 mg. N/kg. respectively. It has been shown elsewhere [Richardson, 1935] that equilibria exist in the ammonia and nitrate contents of grassland soils; the equilibrium levels in the young grassland soil of the present experiment were lower than those observed in older grassland soils.

Incubated soils

For incubation, 100 g. of fresh soil from each sample were placed in a 350 ml. flask, along with a short test-tube containing 12 ml. of N KOH, and the stoppered flask was placed in an incubator at 30°. On the following day, when the moisture content of the fresh soil had been determined, sufficient water was added to make up the moisture content to 20% on a dry soil basis. Air was blown into the flasks at 2-3-day intervals to maintain the oxygen supply; blank

flasks containing KOH tubes were similarly treated, but the correction necessary for CO₂ in the air was small. After 3 weeks' incubation the flasks were removed, and absorbed CO₂ was determined in the KOH by double titration, first to pH 8.4 (phenolphthalein) and then to pH 3.9 (bromophenol blue) [Orchard, 1933]. Ammonia and nitrate were determined in the soil by the procedure already described. The results are given in Table VIII.

Table VIII. *Ammonia and nitrate nitrogen in incubated soil; carbon dioxide produced during incubation*

Sampling date	...	April	May				June				July
		25	7	11	19	26	3	9	16	22	30 ⁶
Ammonia N, mg./kg. (mean of 4)		0.9	2.2	1.0	0.5	0.7	1.1	0.4	0.4	0.8	0.6
s.e. of mean							±0.18				
Nitrate N, mg./kg. (mean of 4)		10.3	14.5	12.0	11.7	9.1	10.6	9.6	10.0	5.5	6.7
s.e. of mean							±1.11				8.2
Carbon dioxide C, mg./kg. (mean of 4)		364	292	274	295	314	358	338	328	342	307
s.e. of mean							±15.8				277

The soil nitrified readily on incubation, with the result that the ammonia values were below those in the fresh soil. The highest ammonia content was only 2.2 mg. N/kg. in the sample of 7 May, taken 3 days after sulphate of ammonia application. There was a significant rise in nitrate content on 7 May, after the addition of sulphate of ammonia, and then a tendency to decrease: for some time the values remained about the level of the initial sample, but at the end of June there was a fall to values significantly below that level.

Carbon dioxide production was very high by comparison with nitrate production, and whereas nitrate increased in the samples taken after sulphate of ammonia application, carbon dioxide decreased. Throughout May it was significantly below the initial value; it rose to a level nearer the initial value during June, but fell again significantly below it at the end of June and the beginning of July.

Some of the fluctuations in the ammonia and nitrate contents of the incubated soils were clearly due to the presence of the nitrogen added as sulphate of ammonia. To eliminate this, the mineral (ammonia plus nitrate) nitrogen found in the fresh soils was subtracted from that in the incubated soils, the difference, known as the "mineralizable" nitrogen, representing the nitrogen made available by the breakdown of soil organic matter during incubation.

The values for mineralizable nitrogen are shown in Fig. 2, the carbon produced as carbon dioxide during incubation being included for comparison. Both curves show fluctuations of the type commonly observed in microbiological studies of the soil, but there are some clearly marked tendencies. The mineralizable nitrogen was decreasing during most of the period of sampling. Such a decrease during the late spring has been observed in other grassland soils [Richardson, 1935] and attributed to a seasonal rhythm in the soil organic matter. As the soil warms up in the spring, the readily decomposable nitrogen in the soil organic matter is mineralized and taken up by the herbage, leaving less mineralizable nitrogen to appear on incubation. The mineralizable nitrogen was low throughout, by comparison with the level found in older grassland soils.

The carbon dioxide values, which may be taken as an index to the amount of readily decomposable carbohydrate material in the soil, show a fall after sulphate of ammonia addition that was probably due to an accelerated breakdown of carbohydrates in the field. Then there was a rise, attributable to the production of root material rich in carbohydrate by the rye grass. Once this ceased, about mid-June (22 June), there was a fall in both carbon dioxide and mineralizable nitrogen. The peak corresponded roughly with that of maximum content of fructosan.

Fig. 2. Changes in the incubation products of rye grass soil. (Note that the vertical scales are different for each of the curves.)

Carbon dioxide and mineralizable nitrogen values frequently appeared to move in opposite directions, as would be expected if some mineral nitrogen was locked up by the micro-organisms that broke down carbohydrates. The major changes in carbon and nitrogen level were not, however, always opposed, and the correlation of the two sets of values, although negative, was not significant ($r = -0.2376$).

The ratio of carbon produced as CO_2 to mineralizable nitrogen, which gives a general index to the condition of the soil organic matter, is also shown in Fig. 2. After a slight decrease resulting from the sulphate of ammonia treatment there was a general rise until the time of cutting hay; evidently the roots, like the shoots, were becoming relatively richer in carbohydrates and lower in nitrogen

content, so that their residues gave a wider carbon-nitrogen ratio in the products of incubation. The cause of the fall in the ratio after 22 June is not so clear; we owe to Dr Nicol the suggestion that there may have been some "resorption" of nitrogen back into the roots from the above-ground parts of the plant.

The soil samples taken from plots cut a week before sampling contained, on the average, slightly more ammonia and gave more nitrate and carbon dioxide on incubation, but the differences from the freshly cut plots were not significant.

SUMMARY

1. The changes in the composition of rye grass cut at weekly intervals throughout the season have been studied and at the same time the amounts of the more mobile forms of nitrogen and carbon in the soil have been followed.

2. The presence of a water-soluble fructosan was confirmed; this constituent increased to a peak of 30.1% at about the time of full emergence of the head, thereafter decreasing with maturity.

3. The fructosan accumulates chiefly in the stem and appears to act as a temporary reserve, later being utilized for the production of structural constituents.

4. The second growth of grass contained a higher proportion of structural constituents and less fructosan than the first growth when compared on a basis of similar protein content.

5. Ammonia and nitrate determinations in the fresh soil showed rapid disappearance of most of the nitrogen added as sulphate of ammonia, with very little production of nitrate.

6. The determination of ammonia, nitrate and carbon dioxide produced by incubated soil samples indicated that nitrification was fairly rapid but that the amount of nitrate or of mineral nitrogen formed was small in comparison with older grassland soils. The soil appeared to become richer in carbohydrate material up to the time of hay cutting and consequently the C/N ratio increased. After this point a steady decrease was observed.

REFERENCES

- Eggleton (1934). *J. agric. Sci.* **24**, 416.
— (1935). *Biochem. J.* **29**, 1389.
Marshall (1936). Thesis, University of London.
Norman (1935). *J. agric. Sci.* **25**, 529.
— (1936). *Biochem. J.* **30**, 1354.
Olsen (1929). *C.R. Lab. Carlsberg*, **17** (15), 12.
Orchard (1933). Thesis, University of London.
Phillips & Goss (1935). *J. agric. Res.* **51**, 301.
Richardson (1932). *Nature, Lond.*, **130**, 96.
— (1935). *Trans. III. Int. Congr. Soil Sci.* **1**, 219.