STUDIES ON THE DECOMPOSITION OF PLANT MATERIAL IN SOIL. I.

LOSSES OF CARBON FROM ^{14}C LABELLED RYEGRASS INCUBATED WITH SOIL IN THE FIELD

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Summary

A new method for determining ^{14}C in soil is described.

Ryegrass roots and tops uniformly labelled with ^{14}C were allowed to decompose for 4 years in soil under field conditions. About one-third of the labelled (ryegrass) C was left in the soil after 6 months but thereafter decomposition was much slower, about one fifth of the labelled C remaining after 4 years. Throughout the period, labelled C was less resistant to decomposition than unlabelled C, i.e. the C present in the soil before the labelled ryegrass was added. Even in the fourth year after addition of the ryegrass, the percentage of labelled C in the soil decomposing per year was four times that of unlabelled C.

Initially, ryegrass tops decomposed more quickly than roots but after 1 year the differences disappeared and the same amount of residual C remained from each. For periods of 1 year and over, the percentage of labelled C retained in a soil with 2·4 per cent organic C was the same as in a soil containing 1 per cent organic C. The percentage C retained was the same when either 0·3 per cent ryegrass tops or 0·6 per cent ryegrass tops were added to the soil. Similar amounts of labelled plant C were retained in soils incubated for 1 year in two contrasting seasons (1959 and 1962).

Introduction

THERE are formidable difficulties in following the decomposition of plant material in soil under natural or near-natural conditions. In mature soils of temperate regions, decomposition continues for many years and the amount of plant C added in any one year is a small fraction of the soil organic C already present. Yet accurate data on decomposition rates are essential to any discussion of the dynamics of soil organic matter in the field.

Losses of plant C in a given time have usually been obtained by measuring the loss of C from soil and plant material incubated together, and then subtracting the loss of C from the soil incubated by itself. This procedure has a serious disadvantage: it is assumed that the addition of fresh plant material does not alter the decomposition rate of the organic matter already present in the soil (i.e. that there is no 'priming action'). Also, when the amount of plant material is kept small relative to the amount of soil organic matter, the analytical errors in measuring its decomposition are large, particularly after incubation periods of more than a few months, when the rapid phase of the attack on plant material is over. When large amounts of plant material are added to reduce the analytical error, the results may not be relevant to the field.

The production of plant material uniformly labelled with ^{14}C has made it possible to follow losses of added plant C accurately, in the presence of large amounts of unlabelled C, for as many years as the

patience of the investigator allows. The newly added C can also be identified as it enters the various fractions of the 'native' soil organic matter.

Parts I, II, and III of the present series of papers are restricted to an examination of the decomposition of uniformly labelled ryegrass under field conditions, and Part IV to its decomposition under controlled conditions in the laboratory. Two soils with widely different organic matter contents were used throughout the work. Both came from adjacent plots of the same experimental field, both contained calcium carbonate (which restricted pH changes during decomposition) and both were sampled after 1 year's bare fallow.

Throughout this series of papers, C derived from labelled ryegrass added to a soil is termed 'labelled' C. In calculating the content of 'labelled' C in a sample from the measured ^{14}C content, it is assumed that the specific activity of the ryegrass-derived C remains unaltered during decomposition: the validity of this assumption is examined in the section on experimental errors. The total C in a sample less the 'labelled' C is termed 'unlabelled' C. In these experiments it comes mainly from the organic matter present in the soil before labelled ryegrass was added.

One aim of the work in this paper was to contrast decomposition rates of ryegrass roots and tops in two soils differing only in organic-matter content, under conditions as near as possible to those occurring in the field. Another was to compare the decomposition rate of the unlabelled soil organic C with that of the labelled ryegrass C over a period of several years. The third, and main aim, was to obtain a range of soil samples that had been incubated with labelled plant material for different times in order to see how the labelled ryegrass C became distributed throughout the unlabelled soil organic matter as decomposition progressed (Parts II and III).

Materials. The soils were from the Broadbalk Continuous Wheat Experiment at Rothamsted; soil I was from the plot (2B) which has received 14 tons of farmyard manure per acre per annum since 1843 except for those years in which it was fallowed (since 1931, once in every 5 years); soil II was from the adjacent plot (3) which has received no manure since 1839. The soils were sampled in September 1958 to a depth of 6 in. from sections of the plots which had lain fallow 1 year, so that no plant remains had been in the soil for less than 1 year. The samples were allowed to dry just to the stage where crumbs no longer adhered under slight pressure, passed through a 2-mm steel sieve, thoroughly mixed, and stored at $-15°$ C in polythene bags until required. Soil I as stored contained 18·7 per cent water and soil II 13·8 per cent, both on an oven-dry basis. Table 1 gives analytical data on the soils. The preparation, analysis, and uniformity of labelling of the ryegrass tops and roots are described elsewhere (Jenkinson, 1960; the analyses in Table 2 of this reference were done on labelled ryegrass roots and tops sampled from those used in the present work). Both roots and tops were freeze-dried and ground to pass a 0·5-mm sieve. Their composition is given in Table 2.

Incubation method. The stored soil was thawed, an amount calculated to contain 1 kg oven-dry soil weighed into a 2 l bottle, mixed with the labelled plant material by end over end shaking, and tipped into an incubation bottle made from a wide-necked bottle 9 cm in diameter and 16 cm long (excluding the neck) with the bottom cut off. A disc of glass cloth was tied over the neck using string made from glass fibre

TABLE 1

Analysis of Soils

	*Composition, % oven dry material**	
	Soil I	*Soil II*
Total C	2·57	1·13
Carbonate C	0·14	0·16
Total N	0·246	0·107
Nitrate N	0·0030	0·0012
Coarse sand	9·4	8·6
Fine sand	41·5	47·4
Silt	22·0	22·5
Clay	18·0	17·5
pH	7·8	8·1
Water held†	32	29

* 24 hours at 80° C. † By the sieved soil under a suction of 10 cm water.

TABLE 2

Analysis of Plant Materials

Material	*Oven-dry matter, %**	*Specific activity* μC/g.C**	*Composition, % oven dry material* C	N†	*Ash*‡
Labelled ryegrass tops	96·7	23·8	43·5	2·09	6·8
Labelled ryegrass roots	96·8	21·0	37·5	1·34	20·8

* 24 hours at 80° C. ** With respect to a standard sodium carbonate source ±5% nominal activity. † Includes nitrate. ‡ 3 hours at 550° C.

and the bottle then set into bare packed soil neck downwards so that the rim projected about 3 cm above soil level. A little sand was placed just below the neck on the outside of the glass cloth, to allow free drainage through the bottle by providing capillary contact between soil inside and outside. The surface of both the labelled soil and the surrounding soil outside the bottles was covered with flints (2–3 cm diam.) to prevent rain from spattering the soil. Soil levels inside and outside the bottles were almost the same. The experiment was sited in an open field away from houses and trees. Both bottles and surround were kept free of vegetation.

At yearly intervals, each soil was removed, sieved moist through a 2-mm steel sieve, sampled and returned to the bottles in the field. When the soil was very wet, it was spread out and allowed to dry (at 10–12° C) just enough to be sieved. Samples at intermediate times were obtained by taking three 12-mm cores to the full depth of the bottle. Samples were stored at −15° C until required.

The treatments, shown in Table 3, were in duplicate. The experiments were started in April 1959. Treatments 1 and 2 were repeated in April 1962.

Analytical methods. Labelled soils were analysed moist and the results expressed on an oven-dry basis by drying duplicate 2 g samples at 80° C for 24 h. This procedure decreased sampling errors and minimized dispersion of radioactive dust. All soil analyses were done in duplicate.

Total C in plant material and soil was determined gravimetrically by a modification of Shaw's (1959) method. Shaw removed carbonates from

TABLE 3

Treatments used in the Experiment

No.	*Soil*	*% added ryegrass*	*labelled mg/C/100 g soil*
1	I	0·3 (tops)	126
2	II	0·3 (tops)	126
3	I	0·6 (tops)	252
4	I	0·35 (roots)	127
5	II	0·35 (roots)	127
6	I	None	None
7	II	None	None

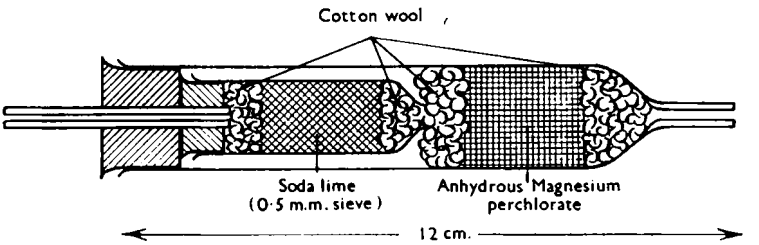

FIG. 1. Absorber for carbon dioxide.

calcareous soils before analysis by pretreatment with H_2SO_3, but as this was not suitable for the present work, carbonate C and organic C were determined together, and organic C obtained by subtracting the inorganic C. To get quantitative recovery of CO_2 from carbonates the following slight modification of Shaw's procedure was used. To the soil sample (usually 1·5 g soil I or 3 g soil II) plus potassium dichromate (3 g) was added 8 ml phosphoric acid (5 parts 88 per cent H_3PO_4+3 parts water), the mixture refluxed 5 min, 20 ml of mixed acid (1 part 88 per cent H_3PO_4+3 parts 98 per cent H_2SO_4) added and the mixture refluxed a further 10 min. The CO_2 was collected in a special absorber, illustrated in Fig. 1, rather than in U-tubes as specified by Shaw, because of the difficulty of removing ^{14}C labelled soda lime quantitatively from U-tubes: two of these absorbers were used in series but normally all the CO_2 was caught in the first. The H_2SO_4 bubbler in Shaw's apparatus was omitted. Recoveries of C from a range of non-calcareous soils were the same as by the unmodified Shaw method. Recovery of carbonate C was complete even from 2 mm grains of Iceland spar or dolomite.

Carbonate C was also determined in Shaw's apparatus by adding cold N HCl (50 ml) to 5 g soil in a 100-ml flask and collecting the CO_2 in the absorption train. The soil was mechanically stirred and air was sucked through the soil suspension for 2 hours to sweep through and collect all the CO_2.

Nitrate and ammonium N in soil were determined by Bremner and Shaw's method (1958), total N in soil by the Kjeldahl method, using copper sulphate and selenium as catalysts. N in plant material was determined by a modification of the Kjeldahl method which includes nitrate-N (A.O.A.C. Methods of Analysis, 1960). pH was measured with the glass electrode using a 1:2 soil:water ratio.

Determination of ^{14}C in soil. A new technique was developed. The soil organic matter was oxidized to CO_2 which was absorbed in tetraethylammonium hydroxide, a dioxan-based phosphor added, and the ^{14}C determined by scintillation counting. Tetraethylammonium hydroxide was introduced because it is non-volatile, and can be used in aqueous solution to absorb CO_2 from soil incubations without influencing soil respiration (see Part II). Both tetraethylammonium hydroxide and carbonate in aqueous solution were sufficiently miscible with the organic phosphor to give a one-phase solution for counting. This solution could hold up to 3 mg of CO_2 per ml. The counting efficiency was about 25 per cent. The principal advantage of this technique was that all the ^{14}C measurements could be put on a routine basis. This could not be done with any of the absorbents used by others (Primene, Hyamine hydroxide, ethanolamine, phenethylamine), because none of these were suitable for all parts of the work in this series of papers, particularly that described in Part II.

The detailed procedure was: the soil was oxidized with acid dichromate by the modified Shaw procedure, the evolved CO_2 estimated by weighing the absorber, the inner absorber tube (Fig. 1) containing the soda lime removed, placed in a 350-ml bottle containing 8 ml of tetraethylammonium hydroxide (25 per cent solution obtained from Hopkin & Williams, Ltd.) in a tube graduated at 10 ml, and stoppered with a bung carrying a two-way tap, one exit being connected to a water pump, the other to a dropping funnel containing 50 ml dil. HNO_3 (1 part 72 per cent HNO_3 + 3 parts water). The bottle was evacuated, the HNO_3 run in, left for 3 days and then the tetraethylammonium hydroxide made up to 10 ml with water.

One ml aliquots were placed in each of two optically matched vials for counting. Six ml of phosphor A was added to one vial and 6 ml phosphor B to the other, the vials allowed to stand overnight in the dark and counted in a Panax SC-LP scintillation counter at room temperature. Phosphor solution A was prepared by making 5 ml of absolute ethanol up to 100 ml with stock phosphor solution C and phosphor B by making 5 ml of an ethanolic solution containing 2-(methyl-^{14}C) naphthalene (solution D) up to 100 ml with stock solution C. Stock phosphor solution C contained 100 g naphthalene (M.A.R. grade), 10 g 2, 5-diphenyloxazole, 0·05 g 1, 4-bis-(2-(5-phenyloxazolyl))-benzene (both obtained from Nuclear Enterprises, Ltd.), 320 ml absolute ethanol and 33 ml

methyl acetate, all made up to 1,000 ml with dioxan (A.R. grade). Solution D was made by dissolving 0·1 mc 2-(methyl-^{14}C) naphthalene in 0·2 ml dioxan, adding 2·5 g naphthalene (M.A.R. grade) and making up to 1,500 ml with absolute ethanol. The amount of internal standard added to a vial gave a count of about 10^4 c.p.m. By using an internal standard with each sample in this way, corrections for quenching of the phosphor are eliminated and the counts obtained can be related directly to the amount of plant C in the sample, because the internal standard had been compared initially with the activity from known amounts of plant material. In making this comparison a known amount (usually 75 mg) of labelled ryegrass tops (or roots) was oxidized, the CO_2 determined gravimetrically and then transferred to tetraethylammonium hydroxide, all as described above, and a suitable aliquot counted with and without internal standard. A tetraethylammonium hydroxide solution of CO_2 from soil incubated without labelled plant material was used to obtain the background count, which was normally about 70 c.p.m. Vials containing internal standard were counted for 2 minutes, and those with sample alone for 5–20 min, the time being chosen to give a total count of at least 5,000 above background. The standard deviation of a determination of labelled C in a soil sample (soil II incubated 1 year with ryegrass tops, 126 mg plant C/100 g soil) was 5·8 per cent (six determinations). Recovery of ^{14}C added to unlabelled soil as Na_2CO_3 solution and then subjected to oxidation, transfer, and counting as above was quantitative within experimental error.

Results

Fig. 2 shows the percentage of labelled C retained by soil incubated in the field. Roots (treatments 4 and 5) were less decomposed than tops (treatments 1, 2, and 3) after 3 months, but by a year the differences had disappeared: for periods of from 1 to 4 years after addition of the labelled ryegrass there are no significant differences ($P = 0{\cdot}05$) between any of the treatments.

Fig. 2 also shows that, for the first 2 years of the experiment, the percentage of unlabelled organic C retained at any one sampling date did not differ significantly ($P = 0{\cdot}05$) between any of the treatments. It follows that the percentage loss of unlabelled C from soil I could not be distinguished from that of soil II over the first 2 years; nor did the addition of fresh plant material measurably alter the decomposition rate of the unlabelled organic matter in either soil. However, the experimental errors in the determination of unlabelled C are large relative to the changes being measured. For example, for the additional loss of unlabelled C to be statistically significant at the 5 per cent level, the addition of 252 mg of ryegrass tops to soil I would have to increase the loss of unlabelled C from the soil during the first year to almost three times that of the soil incubated alone. Three years after the start of the experiment one of the two remaining treatments using soil II retained significantly more unlabelled C than any of the other treatments, and at the fourth year the other remaining treatment using soil II retained significantly more than any of the other treatments. Probably if the

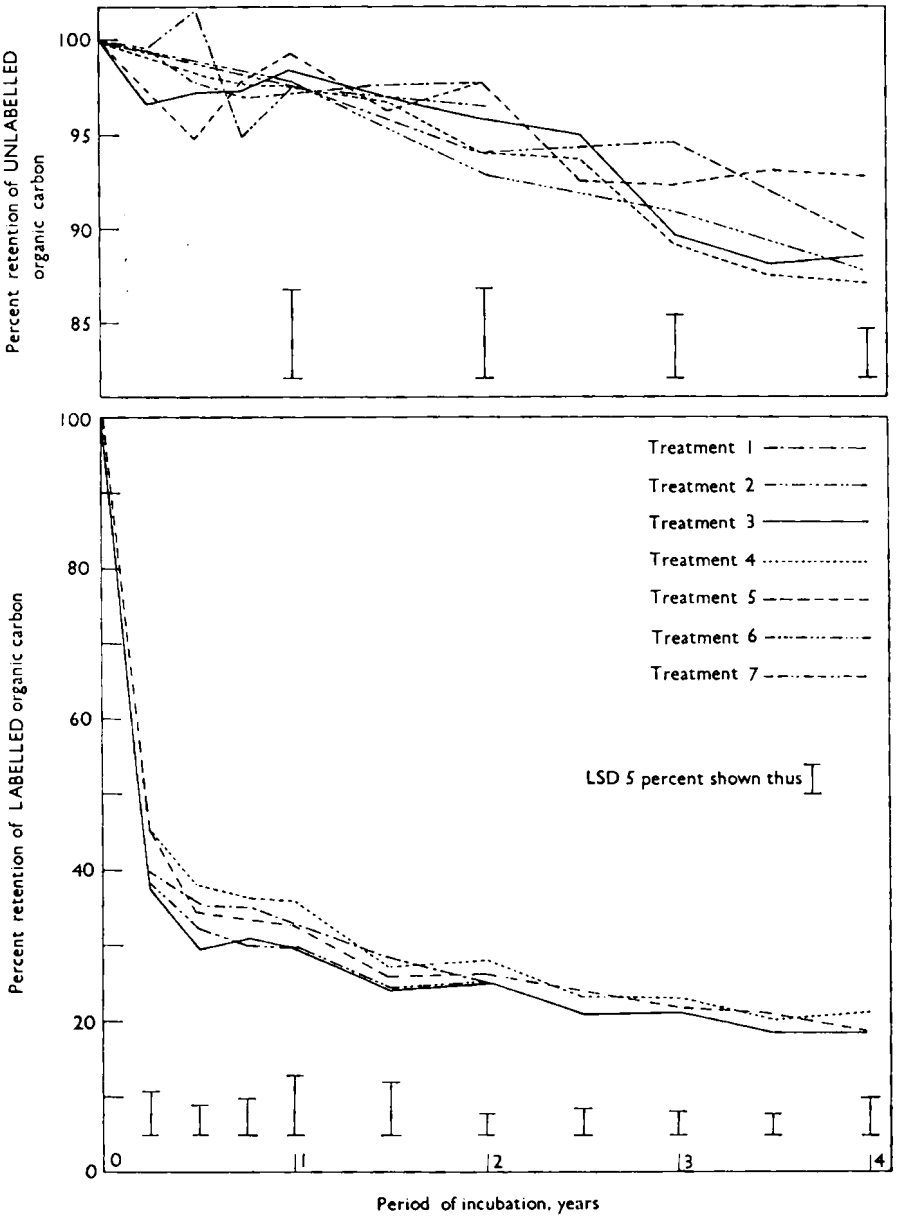

FIG. 2. Retention of labelled and unlabelled C in soils incubated in the field with labelled ryegrass.

experiment had continued, the decomposition rate of the unlabelled organic C in soil II would have been shown unequivocally to be less than that in soil I.

Table 4 gives the results of incubations started in April 1962 to check the reproducibility of the incubations started in 1959. Although April

1959 was followed by a hot dry summer and a mild winter, whereas April 1962 was followed by a cool summer and a severe winter, there were no significant differences (P = 0·05) between the amounts of labelled C retained in the soils.

Experimental errors. Fig. 2 shows values for the Least Significant Differences (P = 0·05) between measurements of labelled C at each sampling date. The standard errors used in calculating the L.S.D.'s include contributions from replicate differences, soil sampling errors, gravimetric and volumetric errors in preparing a sample for counting from a known amount of soil, and counting errors. In addition a systematic error, common to all labelled C results, is introduced by errors in

TABLE 4

Labelled C retained in Soils incubated with Labelled Ryegrass in 1959 and 1962

Decomposition period, years	*% Labelled C remaining in soil*			
	*Soil I**		*Soil II**	
	1959	*1962*	*1959*	*1962*
0	100	100	100	100
0·5	35·4	33·3	32·2	33·0
1·0	32·7	32·4	29·8	31·0

* Incubated with ryegrass tops, 126 mg plant C/100 g soil.

the initial comparison of the standard methyl naphthalene source with the counts from a known quantity of plant C. The standard error of this comparison (four replicate determinations) was ±1·9 per cent.

Errors are also introduced in assuming that the retention of ^{14}C by a soil is directly proportional to the retention of plant C. This implies that the plant material is uniformly labelled initially and that no isotopic fractionation occurs during the decomposition process. Data on the specific activity of different fractions of labelled ryegrass tops (Jenkinson, 1960, table 2) show that the specific activity of certain fractions of the plant material differs from that of the unfractionated material. If the labelled C left in soil I after, say, 1 year's incubation with ryegrass tops, comes exclusively from the fraction of greatest specific activity (water-soluble fraction), the calculated amount of labelled C remaining in the soil would be 5·4 per cent high; if from the fraction of least specific activity (cellulosic fraction) 3·4 per cent low. However, Chekalov and Illuvieva (1962) showed that both water-soluble and cellulosic fractions contribute to the residual C so that these errors will tend to cancel, and the overall error from this source is probably not greater than 2 or 3 per cent of the labelled C retained.

Isotope fractionation during the decomposition of organic matter was considered by Craig (1954) who showed that no fractionation of ^{13}C (and hence presumably ^{14}C) occurred during the decay of wood. Although it is improbable that isotopic enrichment could operate during the decay of solid polymeric material, enrichment could take place during the microbial fixation of CO_2 (see, for example, Sorokin, 1960). How-

ever, so little of the soil organic C originates in this way that fractionation by this mechanism will be undetectable by the methods used in the present work. This conclusion is consistent with Broecker and Olson's (1960) findings that the $\frac{^{13}C}{^{12}C}$ ratios of several soil organic matter samples fell inside the range of values characteristic of terrestrial plants.

Discussion

In these prolonged incubations the retention of labelled C is not influenced by differences between soils I and II in organic-matter content or in soil features such as water-holding capacity, percentage pore space, and structural stability, which depend greatly on the organic-matter content of a soil. Simonart and Mayaudon (1961) suggested that soil organic matter itself may 'stabilize' (i.e. increase the retention of) some of the organic C in decomposing plant residues. Although the additional organic matter in soil I has not increased the retention of labelled C, the results do not necessarily disprove their suggestion, because both soils I and II may contain enough sites for retention of all the labelled organic C capable of being retained. Soils I and II, with their differing fertility levels (for example soil I produced 28 p.p.m. mineral N when the air-dried unstored soil was incubated moist for 21 days at 25° C, compared with 15 p.p.m. from soil II), retained the same amount of labelled C, which suggests that decomposition is not being restricted by shortage of microbial nutrients.

Doubling the addition of ryegrass tops to soil I does not change the fraction decomposed in a given time. This result is in accord with the findings of a more extensive series of laboratory experiments on the effects of rate of addition on decomposition and will be discussed with them in Part IV.

During the first few months of the experiment more labelled C was lost from ryegrass tops than from roots. This is probably due to differences in the composition of roots and tops: the percentage of hot water-soluble C is larger in the tops (29·4 per cent) than in the roots (22·2 per cent). However, during the later stages of decomposition the differences between roots and tops disappear. In these experiments Joffe's categorical statement in his review on green manuring (1955) that 'roots decompose much more slowly than top growth ploughed under' holds only for the early stages of decomposition. These field incubations are also experiments on green manuring because the ryegrass additions, cut just before the flowering stage, were green and succulent. Contrary to the widely held theory that such succulent plant materials decay so rapidly that little C remains in the soil after a few months, 33 per cent (average of treatments 3, 4, and 5) of the ryegrass C still remained in the soil after a year, and 19 per cent (average of treatments 3, 4, and 5) after 4 years.

The losses of labelled C (mean of all results in Fig. 2) are plotted on a logarithmic scale in Fig. 3. Labelled C is lost rapidly during the first few months, and thereafter the rate of loss slows greatly. Two broken

lines have been drawn on Fig. 3, one showing the loss of C from soil by a first-order process with a half life of 25 years and the other by a similar process of half life 4 years. The slope of the former approximates roughly to that of the line representing the loss of unlabelled C from soil over

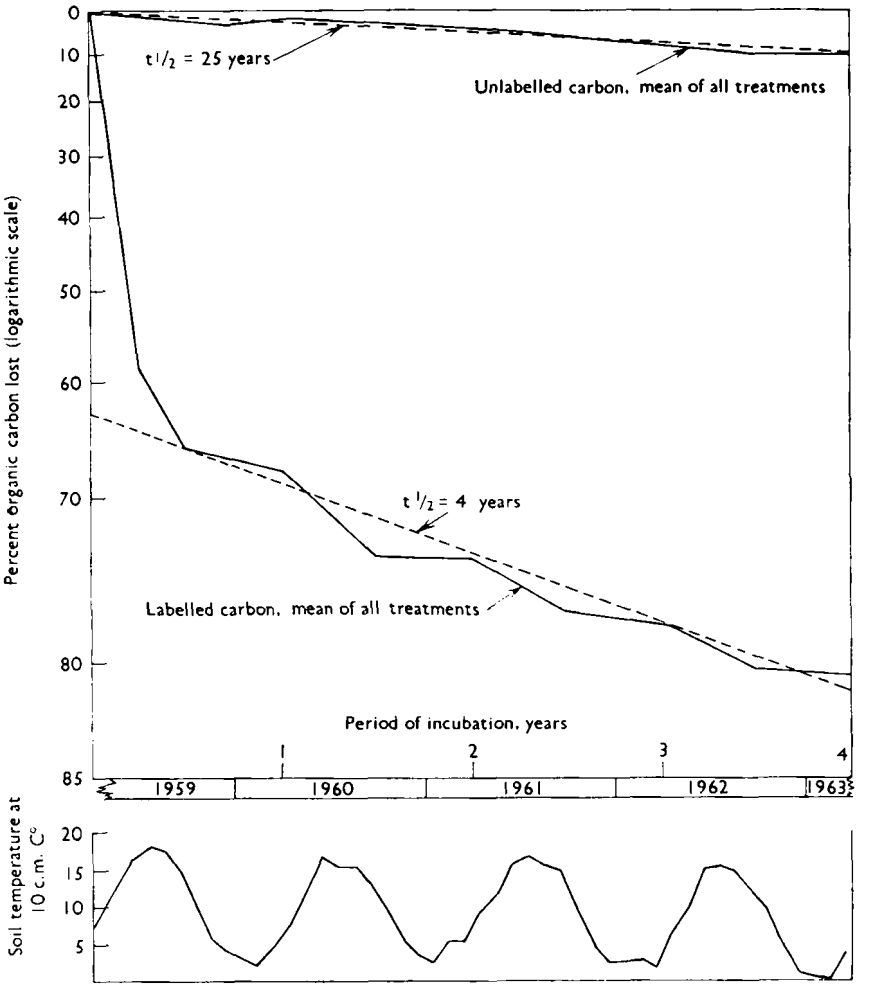

FIG. 3. Losses of unlabelled (soil) and labelled (ryegrass) C from soils incubated in the field with labelled ryegrass.

the whole 4-year period, and the slope of the latter to that of the line showing loss of labelled C over the period 6 months–4 years. Presumably after many years the slope of the decomposition curve of the labelled material will decrease at least to that of the unlabelled soil organic matter, as shown on Fig. 3. Indeed it will probably decrease still further, because the unlabelled soil organic matter contains plant-derived C added as little as 1 year before the start of the experiment, C which is presumably being lost more rapidly than that from older additions.

Table 5 shows the percentages of labelled and unlabelled C lost annually. The ratios of these percentages are also shown: in calculating them the annual percentage of unlabelled carbon lost was averaged over the 4 years of the experiment, because of the large errors involved in measuring individual annual values. Although the figures in Table 5, particularly the ratios, are rough approximations, nevertheless they show that throughout the experiment the added labelled C is more subject to loss than the unlabelled. Even in the fourth year the percentage loss of labelled C was about four times that of the unlabelled C: humification of the added plant material is still far from complete after 4 years in the field.

TABLE 5

Annual Loss of Labelled and Unlabelled Organic C from Soil incubated in the Field with Labelled Ryegrass

Year	*Percentage of C in soil at beginning of year lost during the year*		*Percentage of labelled C lost annually* *Percentage of unlabelled C lost annually*†
	*Labelled***	*Unlabelled***	
1st*	68	2·1	23·8
2nd	19	2·8	6·8
3rd	15	4·0	5·3
4th	12	2·4	4·1

* Labelled ryegrass added at beginning of 1st year.
** Mean of all results in Fig. 2.
† Mean of annual percentages of unlabelled C lost over the first to the fourth years.

The stepped nature of the decomposition curve for plant-C (Fig. 3) indicates that the rate of decomposition over the period April–September is greater than during the period October–March. The mean monthly soil temperatures 10 cm under bare fallow are plotted on the lower part of Fig. 3 for comparison. These were taken from the records of the meteorological station sited about 200 metres from the incubation site. The mean soil temperature 10 cm under bare soil for the 6 months April–September was 13·6° C and the corresponding figure for the period October–March 4·5° C.

All the decomposition experiments discussed in this paper were done under bare fallow and only one addition of plant material was made per treatment. Further field incubations are in progress to compare the decomposition rates of labelled plant material under fallow and under conditions where unlabelled plant material is continuously entering the soil.

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(Received 9 January 1964)