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CHLOROPHYLL-TYPE COMPOUNDS IN SOIL

I. THEIR ORIGIN

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INTRODUCTION

Chlorophyll-type compounds in soil have been little studied but much has been done on those in marine and lake sediments and a general account of these was given by Vallentyne [7]: Chlorophyll *a* occurs extremely rarely in sediments, chlorophyll *b* is more common in some near-surface sediments, but most of the sedimentary green pigments seem to be transformation products of known chlorophylls.

Chlorophyll in soil was reported in 1939 by Torstensson *et al.*[5], who attributed the reddish fluorescence under ultra-violet light of extracts of gyttja-containing soils to chlorophyll compounds. Gorham [1] estimated, by absorption at 667 mμ, the amount of chlorophyll and its derivatives in the 1–2 cm depth of woodland soils in England, and Kumada and Sato [2] suggested that a fraction of humic acids with absorption similar to some porphyrins was derived from chlorophyll in fallen leaves.

The purpose of the following study was to see whether chlorophyll-type compounds in soil indicate the presence of fresh organic matter and if this, in turn, may be related to the amount of nitrogen that the soil may supply to plants, and this paper discusses the sources of chlorophyll-type compounds in the soil.

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ANALYTICAL METHODS

Methods used for measuring chlorophyll

The chlorophyll-type compounds were extracted by shaking with 90 per cent aqueous acetone for 5 hours and then filtering. The soil: extractant ratio was 1:3.

The absorption peak in red light was used to determine the quantity of the compounds; it was invariably between 660 and 670 mμ. The baseline used to eliminate background absorption was the mean of the minimum absorption reading preceding 665 mμ (530 mμ for plants and 630 mμ for soils) and the reading at 750 mμ. The baseline value was subtracted from the absorption peak reading to give the quantity of chlorophyll-type compounds.

Storing the extracts in the dark did not change the absorption values; there was an apparent loss in absorption because of loss in background absorption but this was cancelled in the subtraction. The absorption values were similar after 6 days storage at either 5° or 25°C in the dark, but light (25-watt incandescent lamp placed from 6 to 9 inches from the extracts) destroyed chlorophyll rapidly and after only 6 days there was little absorption at 665 mμ.

The absorption was meaured with a Unicam SP 500 spectrophotometer, using glass cells with either a 1-cm or a 4-cm light path, depending on the concentration of the extract.

Vallentyne [6] introduced the term 'sedimentary chlorophyll units' as an arbitrary measure of chlorophyll degradation products in lake sediments. In the present study the term "chlorophyll units (CU)" is used with the following modifications: (1) CU are calculated from the volume of extractant used and not from the volume of filtrate; (2) CU are calculated from the absorption peak reading near to 665 mμ, after correcting for background absorption, instead of from the absorption peak alone.

A chlorophyll unit is defined here as a fraction of the absorption peak reading, after subtracting background absorption, using cells with a 1-cm light path and a blank of pure extracting solvent. One chlorophyll unit is defined as giving an absorption reading of 0.01 when dissolved in 100 ml of solvent. The following calculation is an example:

> 75 ml of solution were used to extract 25 g of soil. The extract had absorption readings at 630, 665 and 750 mμ of 0.07, 0.20 and 0.01, respectively (measured in a cell with a 4-cm light path).
>
> Absorption by "chlorophyll" (Chlorophyll-type compounds)
>
> $$= 0.20 - \frac{0.07 + 0.01}{2} = 0.16$$

$$\text{CU/100 g soil} = \frac{\text{Absorption by 'chlorophyll'} \times \text{extractant (ml)}}{\text{light path of cells (cm)}} \times \frac{100}{\text{g of soil}}$$

$$= \frac{0.16 \times 75}{4} \times \frac{100}{25} = 12$$

Chlorophyll-type compounds, expressed as percentages of dry weights, were calculated from a specific absorption coefficient (SAC) and from the same absorption readings used to calculate CU. Mackinney [3] found that, in 100 per cent acetone extracts of several commonly grown plants, the SAC of chlorophylls *a* and *b* were 84 and 52 respectively; the SAC of 76 used here assumed a 3:1 ratio of chlorophyll *a:* chlorophyll *b*. Any differences between SAC of chlorophyll and of chlorophyll derivatives were ignored in estimating the weight of chlorophyll.

The relationship between SAC and its component measurements used here is expressed by the equation (SAC) = D/dC, or C = D/(SAC) (d), where C is the concentration of 'chlorophyll' in g per litre, D is the absorption by 'chlorophyll' and d is the light path of the absorption cell in cm. An example of such calculations is:

> 100 ml of solvent were used to extract 0.1 g dry matter of grass; the absorption by 'chlorophyll' was 0.60 (measured in a cell with a 1-cm light path). Because C is in 0.1 litres:

'Chlorophyll' (C) =

$$= \frac{\text{Absorption (D)} \times 0.1}{\text{specific absorption coefficient (SAC)} \times \text{light path of cell in cm (d)}}$$

$$= \frac{0.60 \times 0.1}{76 \times 1} = 0.0008 \text{ g in } 0.1 \text{ g of grass}$$

$$= 0.8\%$$

Development of chlorophyll extraction methods

There is little information on extracting chlorophyll-type compounds from soil, and these are usually in very small amount, so tests were made to find extracting techniques that would give the most concentrated extract without losing accuracy. A clay soil from Rothamsted and a sandy soil from Woburn were used, after being air dried and passed through a 2-mm sieve. The plant material, Italian ryegrass, was air dried and ground to pass a 1-mm sieve.

Samples of soil (33.3 g) and of grass (0.5 g) were each shaken for 5 hours with 100 ml of extractant containing differing proportions of acetone between 70 and 100 per cent. Most chlorophyll units (CU) were extracted from a soil by 85% acetone (Table 1). The concentration was less critical with grass, but most CU were extracted by 80% acetone.

Samples of soil (25 g) alone and mixed with grass (0.375 g), were extracted with 90% acetone by shaking for 5 hours using soil: extactant ratios of 1:2, 1:3 and 1:4. The ratio affected CU extracted from soils alone much more than from the soil–grass mixture (Table 2), but with both soils, either when alone or mixed with grass, the 1:3 ratio gave the most CU, followed by the 1:4 and 1:2 ratios in that order.

Samples of soil (25 g) and samples of grass (0.375 g) were each extracted with 75 ml of 90 per cent acetone by shaking for 1, 2, 3, 4, and 5 hours on an "end-to-end" shaker and then filtering immediately. The number of CU

TABLE 1

Chlorophyll units measured in soil (100 g) and in grass (1.5 g) at varying amounts of acetone to water in the extractant							
Material extracted	Per cent of acetone in the extractant						
	70	75	80	85	90	95	100
Clay soil	2.52	3.30	3.78	3.96	3.84	3.36	2.24
Grass	240	245	248	238	232	235	208

TABLE 2

The effect of soil: extractant ratios on the chlorophyll units extracted from soil alone (100 g) and from a mixture with grass (100 g soil + 1.5 g grass)			
Material extracted	Soil: extractant ratio		
	1 : 2	1 : 3	1 : 4
Clay soil	6.32	7.04	6.80
Clay soil + grass	240	244	242
Sandy soil	2.88	3.92	3.36
Sandy soil + grass	245	249	248

TABLE 3

The effect of shaking time on chlorophyll units extracted from soil (100 g) and from grass (1.5 g)					
Material extracted	Hours of shaking				
	1	2	3	4	5
Clay soil	5.12	6.32	6.72	6.88	6.88
Sandy soil	3.12	3.20	3.28	3.20	3.44
Grass	219	222	222	230	234

extracted from the clay soil increased with times up to 4 hours (Table 3). Duration of shaking had less effect with the sandy soil and with grass.

Samples of soil (33.3 g) and of grass (0.5 g), were extracted alone and mixed. They were extracted initially by adding 100 ml of 90% acetone, shaking for 5 hours and then filtering 50 ml of the solution through a Whatman No. 1 filter paper. The samples were extracted 3 times more by adding 50 ml of 90 per cent acetone to each container, shaking for 30 minutes, and filtering 50 ml of the solution.

The lines in Figure 1 are the number of CU that would be expected from diluting the initial extracts, with which the CU measured in successive extracts agree well. The first and second 25-ml lots of filtrate contained the same number of CU, so any portion of a single extract can be used to calculate the total number of CU in the material.

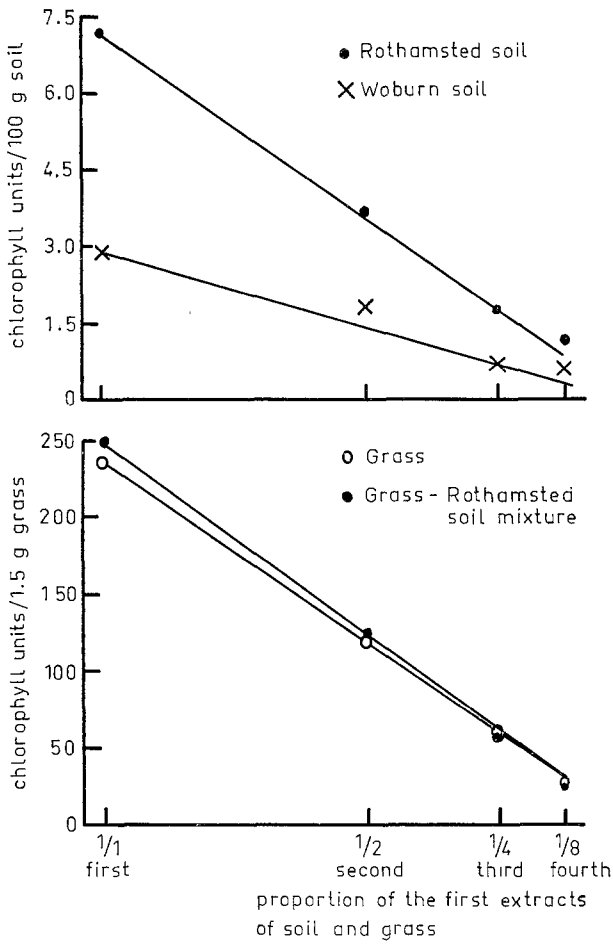

Fig. 1. Chlorophyll units measured in four successive extracts of soil and grass.

EXPERIMENTAL AND RESULTS

Depositions of chlorophyll-containing materials on and in soil

Lucerne and Italian ryegrass were harvested in early June. Potato and sugar beet tops and cereal straw (6-inch stubble) were collected in early

October. Beech and oak leaves were collected in October when about half the leaves had fallen from the trees. Cypress and yew leaves were collected in mid-December from plastic sheets placed under the trees 4 weeks previously. These materials were extracted the same days they were collected, except for cereal straw which was extracted about 2 weeks later.

Fresh faeces of cattle and sheep (less than 12 hours old) were collected in August on pasture, mainly grass; they were extracted on the same day. Farmyard manure (FYM) was sampled from the same heap three times during one year.

TABLE 4

The quantity of chlorophyll-type compounds in various materials normally deposited on, or mixed into the soil

Material		Chlorophyll units per g of dry matter	Chlorophyll % of dry matter
Botanical name	Common name of material extracted		
Lolium perenne	Ryegrass (Italian)	734	0.97
Medicago sativa L.	Lucerne	1,407	1.85
Solanum tuberosum L.	Potato tops	11	0.025
Beta vulgaris L.	Sugar beet tops	269	0.59
Avena sativa L.	Oat straw	5.9	0.013
Hordeum distichnon L.	Barley straw	1.2	0.003
Triticum aestivum L.	Wheat straw	0.5	0.001
Fagus sylvatica L.	Beech leaves	70	0.15
Quercus robur L.	Oak leaves	8.5	0.019
Chamaecyparis lawsoniana	Cypress leaves	40	0.088
Taxus baccata L.	Yew needles	84	0.18
	Cattle faeces	557	1.22
	Sheep faeces	682	1.50
	Farmyard manure (FYM)	14	0.031

Table 4 shows that the amounts of chlorophyll-type compounds in different common plant materials that may be added to soil differ considerably. Italian ryegrass and lucerne contained most and cereal straw least. Beech leaves, which were partly green, contained more than did the oak leaves, which were brown. Cypress and yew contained appreciable amounts, even though they were exposed to light and rain during the 4 weeks they were accumulating.

Fresh cattle and sheep faeces contained quantities of chlorophyll-type compounds similar to those in freshly-cut grass and lucerne. FYM contained only about 5 per cent as much as fresh animal faeces, there was approximately 0.6 lb in a ton of dry matter from FYM.

Decomposition of chlorophyll in excised leaves

Experiment 1. Decomposition in ryegrass leaves by tissue enzymes and micro-organisms. – Leaves of Italian ryegrass harvested when the plants were 6 inches high, were cut into 0.5–1.0 cm lengths. One of two portions of the chopped leaves was placed in boiling water for 7 minutes. Half of each of the samples of boiled and unboiled leaves was ground finely with quartz sand by mortar and pestle. For each of the four treatments, the equivalent of 1 g of fresh grass was placed in each vial, and the vials were put in desiccators containing water in their lower compartments (hygrostats). Incubation at 25°C started one day after harvesting the grass. The moisture contents of the unboiled chopped grass after 0, 6, 18, 42 and 90 days of incubation were 87, 87, 71, 43, and 54 per cent respectively.

Experiment 2. Decomposition in leaves from different species by tissue enzymes. – The leaves of Italian ryegrass, lettuce, oak, holly and yew were collected in early October. The grass leaves were cut into 0.5-cm lengths, the lettuce, oak and holly leaves into 1 cm × 1 cm portions and the needles of yew were stripped from their stems. Incubation started in hygrostats at 25°C on the day the leaves were collected.

Experiment 3. Decomposition in ryegrass leaves in humid and drying conditions. – The leaves of young Italian ryegrass were harvested, cut into 0.5 to 1.0-cm lengths and incubation started, all within half an hour. Some grass was incubated for 6 days at 25°C in hygrostats and some in desiccators with silica gel instead of water in the lower compartment.

Experiment 4. Effect of temperature and water-logging on decomposition of ryegrass leaves. – The leaves of young Italian ryegrass were cut into 0.5–1.0 cm lengths and a portion was boiled for 7 minutes. Within 3 hours of collecting, the boiled and unboiled grass in vials was subjected to 3 different environments (Table 7) for 6 days when chlorophyll was extracted. At the same time, other vials of grass were transferred to aerobic conditions in hygrostats, incubated at 25°C for a further 6 days, and then extracted.

Figure 2 shows that chlorophyll in chopped ryegrass decomposed much faster than in boiled or ground grass; boiling apparently inactivated tissue enzymes. Whether grinding affects tissue enzymes has not been investigated by others, and grinding the leaves may have lessened activity. However, as chlorophyll decomposed in the unboiled ground grass about twice as fast during the first 18 days as in the boiled ground grass, the enzymes were still slightly effective after grinding.

The rapid decomposition in chopped ryegrass leaves was equalled in lettuce and oak leaves (Table 5). There was only a little decomposition for the first 6 days in holly leaves and none in yew leaves. Leaves in which decomposition was rapid were all from plants that were either annuals or deciduous.

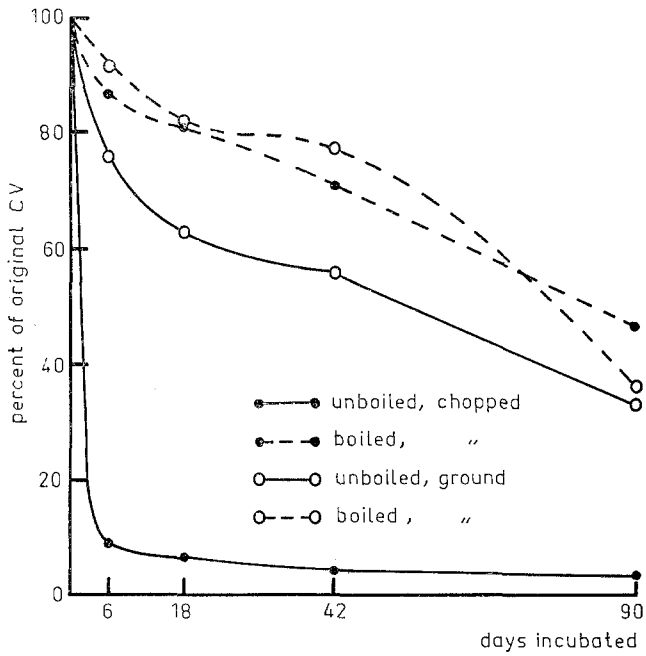

Fig. 2. Decomposition of chlorophyll in ryegrass treated in four ways.

TABLE 5

Chlorophyll decomposition in excised leaves from different species			
Species	Leaves at 0 days		Leaves after 6 days
	Moisture %	CU/g of dry matter	Percent of original CU
Ryegrass	84	777	7.8
Lettuce.	92	521	10
Oak	54	325	42
Holly	67	500	90
Yew	70	487	101

Table 6 shows decomposition in ryegrass leaves in humid and drying conditions during 6-days. When humid the grass lost 90 per cent of its initial CU after 4 days, with no further loss during the next 2 days. Similar material incubated in dry air lost chlorophyll at the same rate during the first 2 days, but then decomposition stopped. At this stage, the leaves still contain 33 per cent water and 53 per cent of the CU remained.

TABLE 6

Enzymic decomposition of chlorophyll in ryegrass under humid and dry conditions

Test period, days	Humid conditions	Dry conditions	
	Percent of original CU	Percent of original CU	Moisture in grass %
0	100	100	78
0.25	97	98	76
0.5	92	94	76
1	84	80	70
2	39	53	33
4	11	52	0
6	10	59	0

TABLE 7

Decomposition of chlorophyll in ryegrass, expressed as per cent of original CU, in three environments for 6 days, and on transfer to aerobic incubation at 25°C for a further 6 days

Time of sampling	In water		At −15°C		At 5°C	
	Boiled leaves	Unboiled leaves	Boiled leaves	Unboiled leaves	Boiled leaves	Unboiled leaves
After 6 days in the 3 environments	68	60	99	101	105	90
After 6 days from transfer to aerobic incubation	74	62	92	85	87	20

Table 7 shows that very little chlorophyll decomposed in boiled chopped ryegrass kept for 6 days at 5°C or −15°C. When kept under water, it lost 32 per cent of its CU and turned brown, suggesting that chlorophyll had been converted to pheophytin, possibly because the contents of the vials had become acid. (About 40 per cent of the absorption near 665 mμ is lost when chlorophyll is converted to pheophytin [4]. When the boiled chopped grass was taken from the 3 different environments and incubated aerobically at 25°C, a small amount of chlorophyll decomposed in the material previously at 5°C or −15°C, but there was no further decomposition in the material kept under water.

In unboiled chopped grass kept in the same conditions, chlorophyll decomposed differently. Some decomposed at 5°C, contrasting

with the boiled material; tissue enzymes seem to be active at 5°C. When transferred to aerobic incubation at 25°C for 6 days, no CU was lost from the grass previously under water and only little was lost from the grass that had been frozen. However, the grass previously at 5°C lost a further 71 per cent of its original CU, so the tissue enzymes were not destroyed by cool storage.

Decomposition and leaching of chlorophyll-type compounds from cattle faeces

Clay soil from Rothamsted sampled 6 inches deep was placed in one of two 10-inch cube boxes stored outside in holes dug in clay soil; the other box contained the same soil acidified with sulphuric acid to lessen microbiological activity. When taken from the field the soil was pH 7.9; after acidifying it

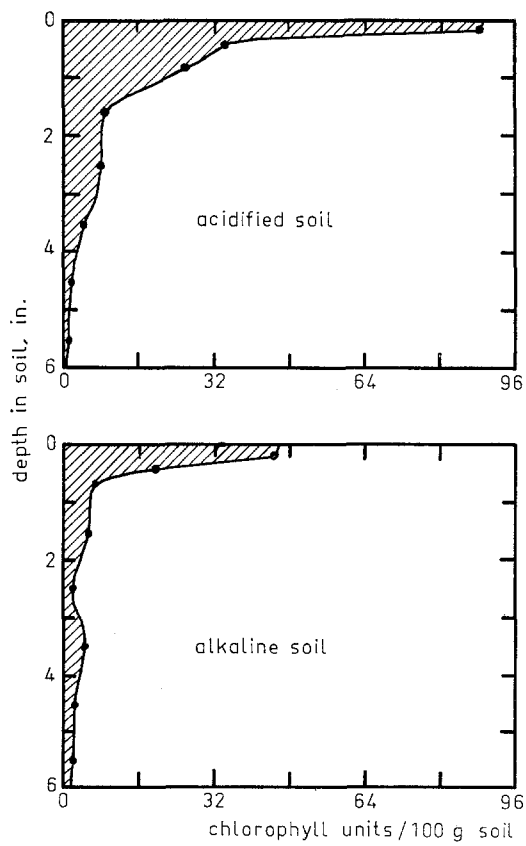

Fig. 3. Vertical distribution in soil of chlorophyll-type compounds leached from fresh cattle faeces placed on top.

was pH 2.6. The soil was placed 6 inches deep in the boxes, which were open at top and bottom, and their tops were at ground level. A nylon mesh net was placed over the soil and a 0.5-inch layer of quartz sand was added. A 2.5-inch layer of fresh faeces (less than 8 hours old) from cattle grazing on pasture was placed on top.

Triplicate 2 g samples of the faeces were taken after 0, 6, 18, 42, and 90 days and their chlorophyll content measured. After 90 days, the nylon mesh nets, the quartz sand and the faeces were carefully removed from the boxes. The soil that had been beneath the faeces was then sampled at 0–$\frac{1}{4}$, $\frac{1}{4}$–$\frac{1}{2}$, $\frac{1}{2}$–1 inches and at successive 1-inch depths to 6 inches, by removing each layer of soil downwards in the boxes. Chlorophyll-type compounds were extracted from duplicate 25 g soil samples from each depth. The period of the experiment was 15 August to 13 November, 1963.

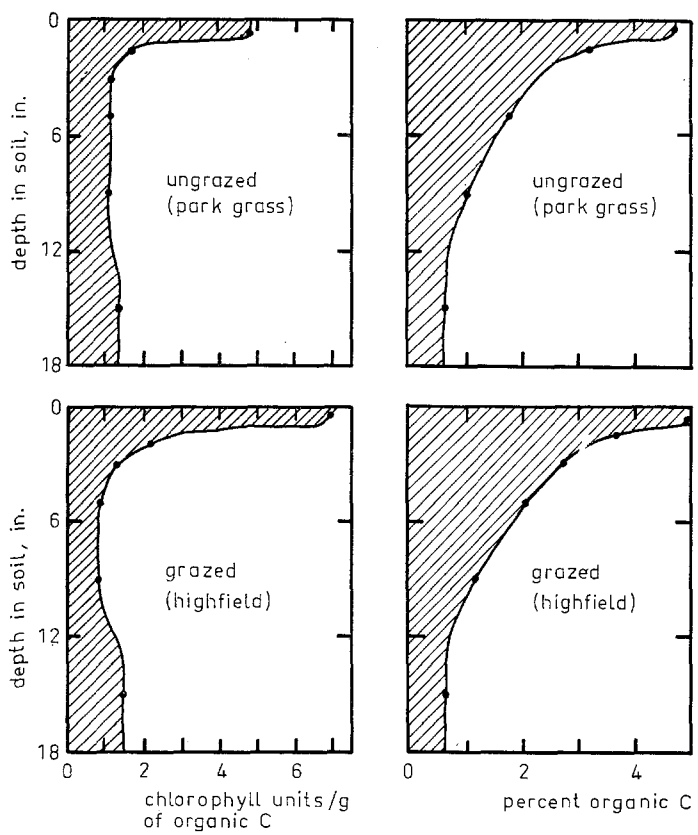

Fig. 4. Vertical distribution of chlorophyll-type compounds and organic C in two grassland profiles.

Chlorophyll-type compounds decomposed very slowly in fresh cattle faeces placed on top of the soil and 78 per cent of the original CU remained after 90 days.

The soil underneath the faeces for 90 days contained chlorophyll-type compounds that had leached from the faeces (Figure 3); the top $\frac{1}{4}$ inch of the acidified soil contained about 90 CU per 100 g soil and although the amount decreased sharply with depth, there was leaching at least 4 inches deep. The soil at pH 7.9, contained about half as much chlorophyll-type compounds as the acidified soil. (Initially the soil contained only 3.2 CU per 100 g soil and this was subtracted to get the results in Figure 3.)

Distribution in soil profiles

Soils were taken from two fields at Rothamsted that have carried grass for over a hundred years. Since 1856, hay has been taken from one whereas the other has mostly been grazed. Samples were taken 0–1, 1–2, 2–4, 4–6, 6–12, and 12–18 inches deep. Most of the chlorophyll-type compounds in these soils were in the top 2 inches (Figure 4). The soil under the grazed grass contained more than under grass cut for hay. Also, there was more organic C under the grazed than under the ungrazed grass.

DISCUSSION

These results are an approximate guide to the quantities of chlorophyll and its derivatives (chlorophyll-type compounds) that enter soil from residues of higher plants. These compounds had partly decomposed in the leaves fallen from trees less than a month before they were collected. In senescent and dead annual plants, the compounds were even more degraded. Tissues containing intact chlorophyll are sometimes incorporated in the soil; examples of this are when green manures or pasture are ploughed in. One ton dry weight of ryegrass, for instance, contributes about 35 pounds. The chlorophyll in these fresh green materials can decompose quickly, for 90 per cent of the 'chlorophyll units' was lost with six days in chopped fresh ryegrass.

The rapid decomposition of chlorophyll in freshly cut, living plants has been attributed to tissue enzymes. Their action was greatly lessened by grinding the leaves finely, and drying, freezing

or water-logging stopped it altogether. In natural conditions, drying is probably the most important factor in arresting and probably destroying these enzymes; mown grass (or any other plant material separated from its roots) dries rapidly when exposed out-of-doors.

In addition to the decomposition of chlorophyll-type compounds by tissue enzymes in fresh intact green material, decomposition is attributed to micro-organisms, and some direct chemical action is also possible. Decomposition by micro-organisms could degrade chlorophyll in material lying on the soil surface for long periods before incorporation.

Cattle and sheep faeces dropped on pasture in summer contained about as much chlorophyll-type compounds per unit of dry weight as growing grass. Chlorophyll units (CU) in cattle faeces were lost only slowly, because decomposition by tissue enzymes seems to be stopped by the passage through the animal's digestive tract, and microbiological decomposition must have been hindered by poor aeration in the faeces. The chlorophyll-type compounds in cattle faeces out of doors leached into soil at least four inches deep during 90 days of autumn weather while 7.2 inches of rain fell. The quantity leached into soil from the faeces was equivalent to that in 1500 lb per acre of fresh grass.

Soil under grass grazed for the past 100 years contained 50 per cent more CU than soil under grass cut for hay, probably because chlorophyll-type compounds leached into the soil from faeces. These compounds must have leached from plant debris or been carried down by soil fauna in the land under hay.

One laboratory experiment (not described here) indicated that the micro-organisms in the arable soils used do not synthesize a measurable quantity of chlorophyll-type compounds. Chlorophyll is synthesized by algae and photosynthetic bacteria, but they form very little in ordinary soils.

In practice chlorophyll-type compounds are added to agricultural soils mainly when leys are ploughed and when domestic animals drop faeces on pasture. (Farmyard manure contains only a twentieth as much as faeces.) Because the chlorophyll in fresh plant tissue, but not in faeces, is destroyed rapidly the faeces are probably a major source of CU in agricultural soils.

SUMMARY

The amounts of chlorophyll-type compounds in materials commonly deposited on or in soil were measured and the processes that destroy them in materials on the soil surface, and the ways they may enter the soil, were studied. Of plant material commonly deposited on the soil, freshly-cut ryegrass and lucerne contained most of such compounds and cereal straw least. Faeces from grazing cattle and sheep contained nearly as mush as grass; farmyard manure contained only five per cent as much as fresh faeces.

Nine-tenths of the chlorophyll in chopped-up, fresh ryegrass leaves was decomposed in six days; this decomposition was attributed to tissue enzymes and was prevented by boiling, drying, water-logging or freezing. Micro-organisms decomposed about sixty per cent of chlorophyll in ryegrass leaves in 90 days.

A large amount of chlorophyll-type compounds in faeces on soil leached 4 inches deep into the soil during 90 days in the autumn. Soil under 100-year-old, grazed pasture contained more of these compounds than under grassland that was cut for hay each year.

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