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THE USE OF $C^{14}O_2$ CANOPY TECHNIQUES FOR MEASURING CARBON TRANSFER THROUGH THE PLANT–SOIL SYSTEM

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SUMMARY

Methods for labelling growing plants by exposing them to $C^{14}O_2$ under a cellulose acetate–butyrate canopy have been developed for laboratory and field use. The length of labelling ranged from 2 to 33 days and the $C^{14}O_2$ content of the atmosphere was automatically controlled. This made it possible to measure carbon assimilation by the plants, transfer of photosynthates beneath ground and respiration of the roots.

In the laboratory, root respiration of wheat plants was measured by separating the above and beneath ground plant parts using a RTV rubber partition. Half to two thirds of the assimilated carbon was found above ground, 15 to 25 per cent in the roots and shoot bases below the partition and 17 to 25 per cent was lost by underground respiration. The variability of these proportions was related to the stage of maturity of the plants.

On native grassland, the relative above and beneath ground productivity was 50 per cent. The time required for the photosynthates to reach the roots at various depths ranged from 1 to 5 days and the amount of material deposited in the roots changed with time and soil moisture content. The use of tubes inserted at various depths beneath the canopy permitted sampling of soil air for C^{14} and CO_2 measurements. The soil $C^{14}O_2$ flux indicated that root respiration during 8 days accounted for 24 per cent of the labelled carbon translocated to the roots after a two days labelling period.

INTRODUCTION

The use of the ecosystem concept and the principal that transfer rates can most easily be expressed in terms of energy, or carbon flow is proving very useful in characterizing the soil–plant complex. Plant growth and microbial activity are closely intertwined in these transfers, and net photosynthesis and soil respiration measurements provide an estimate of the carbon flow under steady state conditions. The quantitative processes involved in photosynthesis, transloca-

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tion of the photosynthates to various portions of the plant, deposition in and turnover of carbon in specific compartments and root exudation must be understood before the system can be characterized.

Radioisotopes such as C^{14} make it possible to tag a portion of the system under study. This has proven very useful for evaluating carbon flow processes in photosynthesis[7] and in the translocation of photosynthates to various portions of the plant [6]. Dahlman and Kucera[2] used $C^{14}O_2$ to label grassland. Sampling at various times after labelling made it possible to determine root turnover rates. In addition, the incorporation of uniformly labelled plant materials or specific biochemicals into soil is being used to measure the dynamics of soil organic matter [4 9].

When the aerial parts of the plant are labelled with $C^{14}O_2$ by photosynthesis, newly assimilated C^{14} can be respired, deposited in these parts of the plant or translocated to the roots. In the roots it can be respired, laid down in the process of growth or exuded with subsequent utilization by micro-organisms. Eventually the assimilated C^{14} will be either consumed by the fauna or decomposed by the microbial population. The long half life, low toxicity and easy handling of the radioactive isotope C^{14} makes it an ideal tracer for determining the overall reaction rates occurring in such factors as growth and root and microbial respiration.

This paper presents techniques for labelling plants with C^{14} under controlled conditions in the laboratory or *in situ* in the field. Methods, for measuring translocation of carbonaceous material within the plant and for ascertaining root respiration rates, using soil sampling tubes and gas chromatographic techniques are also detailed.

MATERIALS AND METHODS

Growing plants were enclosed for several days in a photosynthesis canopy containing $C^{14}O_2$. Labelling experiments were conducted in 1969 and 1970 in the laboratory and at the Matador site of the Canadian IBP. The vegetation is an Agropyron-Koeleria grassland association and the soil a Sceptre heavy clay, classified as Rego Brown Chernozemic. For the growth chamber experiments, the upper 15 cm of the same soil under cultivation were sampled and used to grow wheat plants.

Photosynthesis canopy and CO_2 collection apparatus

A double compartment system was used in the growth chamber. The upper chamber, a cylindrical photosynthesis canopy was made of cellulose acetate-

butyrate of 1.5 mm thickness. This was glued to the lower compartment consisting of a cylindrical polyethylene container which enclosed the soil and root system. An air tight partition separated the two compartments. The stems of the plants emerging through holes made in the partition were sealed with an RTV silicone rubber [10].

The small air space located between the soil and the partition contained inlet and outlet ports such that CO_2-free air could be circulated above the soil. Soil CO_2 was collected in an NaOH trap placed in the outgoing air stream. Sampling tubes, inserted at different depths in the soil, through the

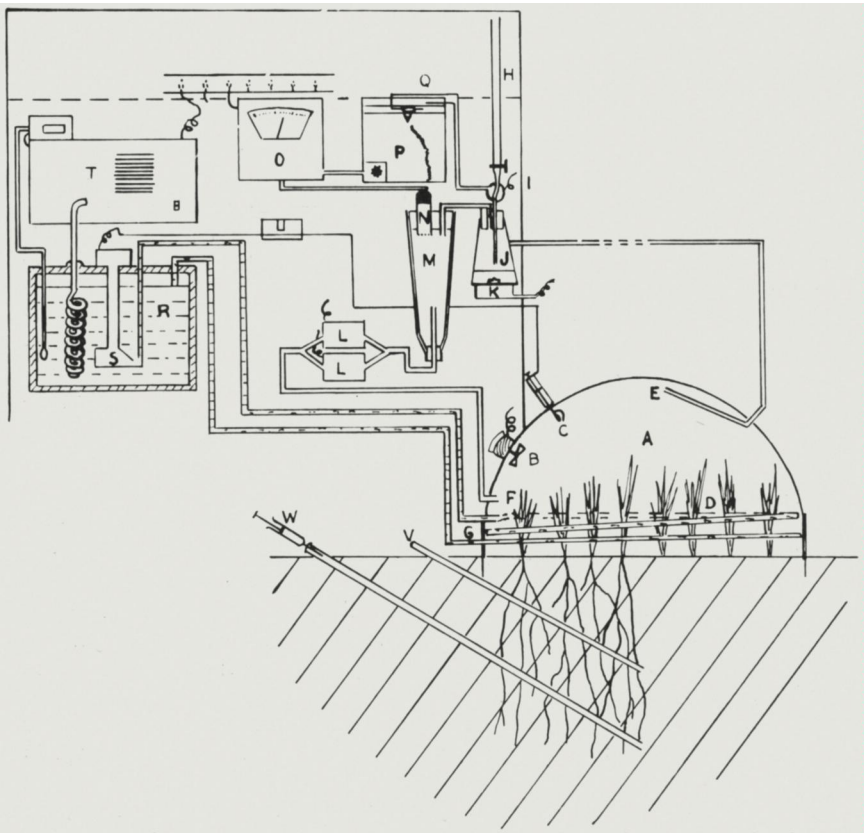

Fig. 1. $C^{14}O_2$-generating, temperature control equipment and photosynthesis canopy used in the field.

A, canopy; B, fan; C, thermistor; D, cooling coil; E, F, air inlet and outlet; G, refrigerant inlet and outlet; H, labelled sodium carbonate buret; I, magnetic valve; J, lactic acid; K, stirrer; L, pumps; M, counting chamber; N, GM tube; O, ratemeter; P, recorder; Q, valve switch; R, refrigerant liquid; S, pump; T, immersion cooler; U, temperature control; V, sampling tube; W, disposable syringe.

container wall enabled sampling of soil air at different depths for measurement of CO_2 concentration and radioactivity.

The canopy for enclosing the native grass in the field consisted of a 40 cm diameter semispherical dome made by heating a cellulose acetate-butyrate sheet under pressure. This was attached to a 10 cm deep steel cylinder driven into the soil (Fig. 1). Aluminum tubes installed at a 45° angle to varying depths under the canopy, permitted the sampling of soil air for CO_2 and radioactivity measurements.

Environmental control equipment

The generation and regulation of the $C^{14}O_2$ and the control of temperature within the system were achieved in the laboratory and in the field by mobile equipment (Fig. 1). The $C^{14}O_2$ was generated by dropping a solution of labelled Na_2CO_3 from a burette into a flask containing lactic acid. Two diaphragm pumps circulated the $C^{14}O_2$ air mixture through the canopy to a counting chamber and back to the CO_2 generating flask. The level of radioactivity in the air was measured by a thin window GM tube mounted in the counting chamber and connected to a rate meter and recorder. This level was kept constant throughout the experiment by a magnetic valve placed on the carbonate buret and activated by a micro-switch from the reading on the recorder.

A refrigerant solution, contained in an insulated cooler connected to a mechanical refrigerating unit, was circulated by a pump through a coil situated inside the canopy. The flow of coolant was controlled by a by-pass valve and circulated when the canopy temperature exceeded the outside temperature as indicated by a thermistor located inside the canopy.

Labelling

In the growth chamber, labelling was conducted on wheat at two different stages of growth at a level of 100 μc/g of CO_2–C during three, 8 hour dark and 16 hour light cycles.

The level of radioactivity used for the native grass in the field was 450 μc/g of CO_2–C and the length of exposure varied between 2 and 3 days. The time of active photosynthesis was dictated by the season; experiments were carried out in June and August.

The following stepwise procedure was commonly used for both laboratory and field experiments: Labelled carbonate (to provide 0.03% of $C^{14}O_2$ v/v in the system) was manually metered through the burette to the flask containing the lactic acid. After the radioactivity had reached an equilibrium level, the controls on the recorder-valve assembly were set to maintain this level of radioactivity. At the beginning of the light period following the cessation of labelling, a solution of unlabelled carbonate was added to maintain normal CO_2 concentration until the radioactivity remaining in the canopy dropped to the natural atmospheric value. The canopy was then removed.

Respiration measurements

Samples of soil air were taken with disposable syringes from the tubes placed at different depths in the soil according to the following procedure: 1-ml sample for CO_2 determination, 10 ml for radioactivity measurements and 1 ml again for CO_2[12]. Data for the two, 1-ml CO_2 determinations were averaged. The sample taken for radioactivity was injected into a scintillation vial sealed with a serum cap and containing 1 ml of 0.2 *N* NaOH under vacuum. The vial was placed on a shaker for 3 hours to ensure adsorption of CO_2 in the NaOH solution. Then 10 ml of Triton-X scintillation liquid [11] were added, and the sample placed in the scintillation counter for activity measurement. For the CO_2 samples taken in the growth chamber and under field conditions where a gas chromatograph was readily available, the tips of the syringes were plugged for transport. Under certain conditions, it was necessary to transport the gas samples from the field to the headquarters laboratory. This was done in 'vacutainers' sealed with serum caps. To ensure accuracy of air removal, the pressure inside the container was adjusted to normal by adding water. CO_2 standards for the gas chromatograph were in this case processed through the vacutainer system.

Carbon dioxide determination and radioactivity measurements made it possible to follow the proportion of labelled carbon respired by the roots throughout the experiments. The length of sampling lasted until this proportion became negligible. In the field, the same data were necessary to calculate the total labelled carbon respiration by the roots using the diffusion method. The fluxes of soil CO_2 and labelled CO_2 at the soil surface were determined according to the calculations of de Jong and Schappert [5]. They have determined the diffusion constant for the soil under study. The flux of gas in g/cm^2 per second is

$$Q = -D_s \frac{dc}{dz}$$

where Q = flux of gas; D_s = diffusion constant in soil, cm^2 per second; c = concentration of gas, g/cm^3 of air; z = depth, cm. Knowing the proportion labelled carbon/total carbon, the flux of labelled carbon was then deducted from the flux of total carbon.

In the growth chamber, the measurement of total and labelled respiration was made by titration of the NaOH solution used to collect the soil CO_2.

Harvesting of plant material

When the level of radioactivity in the soil had dropped to a low value, the above ground parts of the labelled plants were clipped and the below ground part sampled. In the field, root materials were obtained by coring with a 6-cm ID hydraulic corer. The cores were separated into 10 and 15 cm sections which were placed on sieves. The roots were then carefully washed by agitation of the sieves immersed in water. Subsamples of the above ground and root material were ground and the carbon content measured using the dry combustion method. C^{14} was measured in an aliquot removed prior to titration of the NaOH used for collecting evolved CO_2.

In the field, since the soil below the labelling area was not enclosed in a container, a knowledge of the dispersion of the root system was necessary to estimate their biomass and labelled carbon content. Experiments done on native grass have shown a high degree of root competition between plants and consequently very little lateral extention [3]. Vertical cracking of the soil which occurs during the dry months also inhibits lateral distribution of the roots. Therefore, it was assumed that by selecting the soil-root cores taken in the middle of the labelling area, only roots of the labelled plants were likely to be found. Average of three such cores containing the highest radioactivity permitted then, by summation, an estimate of the total labelled carbon located below the ground.

RESULTS

A. Labelling in the growth chamber

The use of a two compartment chamber to enclose the plant–soil system made it possible to calculate the distribution of the assimilated labelled carbon in the plant parts after the period of exposure. The inclusion of the partition prevented soil-CO_2 from being reincorporated by photosynthesis and also enabled measurement of the respiration occurring below the partition.

TABLE 1

Distribution of total carbon and labelled carbon in plant parts, and respiration after exposure to $C^{14}O_2$ for $3\frac{1}{2}$ days followed by 4 days in normal atmosphere (growth chamber experiment)

	Wheat (heading stage)				Wheat (dough stage)			
	Biomass –C mg	Labelled –C mg	Labelled –C mg/g C	Labelled –C distribution %	Biomass –C mg	Labelled –C mg	Labelled –C mg/g C	Labelled –C distribution %
Shoots								
Above partition	4,940	720	146	52	10,990	745	68	69
Shoot bases								
Below partition	1,020	204	200	15	2,800	60	21	6
Roots	3,010	140	46	10	8,880	83	9	8
Respiration								
Below partition	—	321	—	23	—	186	—	17
Total	8,940	1,385	—	100	22,670	1,074	—	100
	Recovery of labelled–C 100%				Recovery of labelled- C 95%			

Incorporation, and root respiration, of labelled carbon are indicated by the data of two labelling experiments on wheat plants at two different stages of growth (Table 1). After 60 days, the plants in the heading stage contained 9 g of carbon, the more mature plants contained almost 23 g. The wheat in the late growth stage, however, contained less labelled carbon (1,074 mg) than the younger plants (1,385 mg). The shoot to root ratio of the labelled carbon was 10 to 1 and 6.6 to 1, respectively. In a cultivated system where the total plant biomass belongs to the growing plant, the amount of labelled carbon per unit of biomass-carbon (specific activity) reflects the relative growth of the different tissues. In the immature wheat, the highest specific activity was found in the shoot bases (200 mg of labelled carbon per g of plant carbon). In wheat at the dough stage, the maximum activity was measured in shoots themselves and was related to the structural development of the seed. Lower values were found for the root tissues.

After removal of the roots by washing, no measurable radioactivity was found in the soil or wash water. This is confirmed by

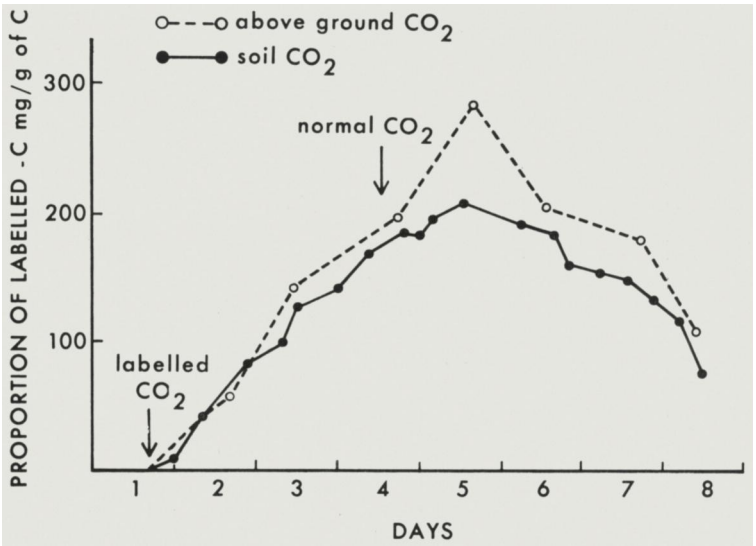

Fig. 2. Proportion of labelled C in soil CO_2 (average of 3 different depth measurements) and above-ground CO_2–C evolved from the soil as a function of time (laboratory experiment – wheat at heading stage). *Arrows* indicate the time when labelled and unlabelled CO_2 were added.

the fact that an average of 97.5 per cent of the added C^{14} was recovered either in the plants or as respired carbon. The low amount of C^{14} in root detritus contrasts with the data of Shamoot *et al.*[8] who found significant amounts of C^{14} in the soil after roots were removed. The labelling in their experiment, however, was conducted over a long period of time in contrast to the short labelling utilized in this study.

The respiration of the labelled carbon by the roots is illustrated in Figures 2 and 3 for the wheat in the heading stage. The specific activity in the soil air (expressed in mg of labelled carbon per g of total carbon) at various depths in the soil container was similar, (data not shown), with the average specific activity being slightly lower than that of the above-ground CO_2 - collected in the NaOH trap (Fig. 2). Labelled carbon appeared approximately 4 hours after initial exposure of the plant to $C^{14}O_2$. The proportion of labelled–C increased linearly with time with a maximum occurring about one

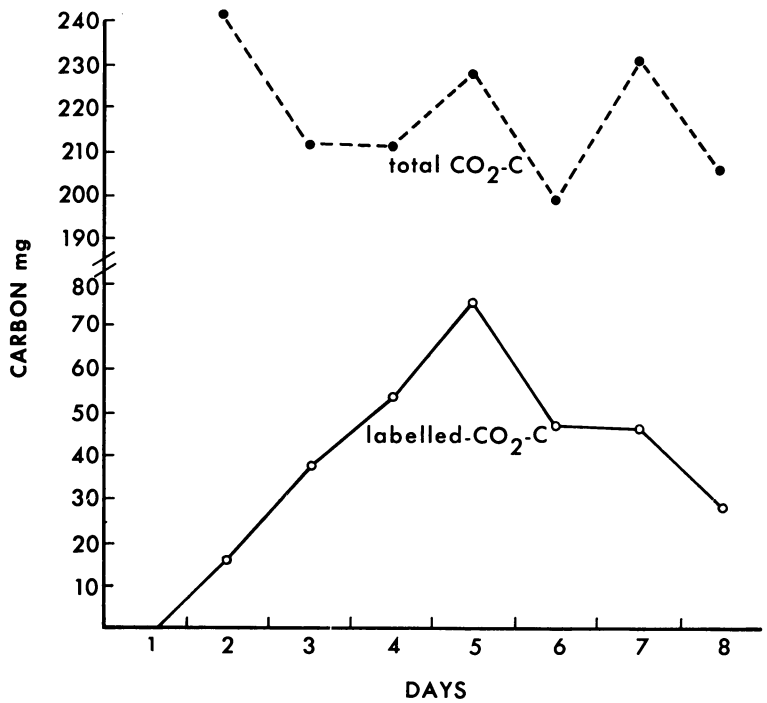

Fig. 3. Daily rates of labelled–C and total–C evolved from the soil as CO_2 (laboratory experiment – wheat at heading stage).

half day after the plants were returned to a normal atmosphere. The lag period indicates the time required for the photosynthates to be translocated from the foliage to the roots where they are metabolized and CO_2 is respired.

The labelling period was not continued long enough to establish steady state conditions between labelled and soil–C. The specific activity of the soil air therefore cannot be taken as an absolute measure of the ratio between beneath ground plant respiration to soil respiration. However, the curve showing the daily rate of labelled CO_2–C (Fig. 3) indicates that the C^{14} respiration was very near completion after the end of 8 days. A total of 320 mg of labelled carbon was respired during this period. This indicates that 90 mg of respired labelled C can be attributed to each day of exposure to $C^{14}O_2$. During each day of this period, the average respired CO_2–C was 215 mg indicating that daily beneath ground plant respiration accounted for at least 40 per cent of the total soil respiration. In this case the shoot bases beneath the partition contribute to the soil plus root respiration.

B. Field experiments

In the field, inclusion of a partition to separate above and below ground portions of the plant–soil system thus preventing photosynthesis of soil CO_2 was impractical during the first years measurements. However, the relative proportion of labelled carbon translocation, distribution within the plant and respiration by the roots, makes it possible to investigate carbon transfer through a virgin ecosystem.

The time required for the labelled photosynthetic products to be translocated from the foliage to different depths of the root system is illustrated by the data obtained from 3 different labellings in August (Table 2). Sampling of the plant material 24 hours after the end of the two days labelling period indicated that 66 per cent of the label was recovered in the shoots. With time, the specific activity of the foliage-carbon decreased and after 120 hours only 46 per cent of the labelled carbon remained above ground. Between 10 and 25 cm, the specific activity of the root tissues reached a maximum after 24 hours (4100 dpm/mg C) and then decreased (3600 and 3000) indicating a continuous movement of translocates downward. Between 25 and 40 cm, the roots showed a maximum of radioactivi-

TABLE 2

Translocation of labelled–C throughout plant parts as a function of time following labelling (field experiments, August)

Sampling time after end of labelling (hrs)		Shoots	Roots and rhizomes 0–10 cm	Roots, depth (cm)				
				10–25	25–40	40–55	55–70	70–85
24	Sp. Activity dpm/mg C	35,500	5,300	4,100	2,400	2,500	3.100	2,100
	Labelled–C distribution (%)	66	22	6	2	2	1.5	0.5
36	Sp. Activity, dpm/mg C	34,900	3,200	3,600	3,300	3,300	4,500	4,800
	Labelled–C distribution (%)	63	18	7	4.5	2.5	2.5	2.5
120	Sp. Activity, dpm/mg C	32,600	5,700	3,000	3,400	3,700	5,400	5,800
	Labelled–C distribution (%)	46	32	6	5	3	4	4

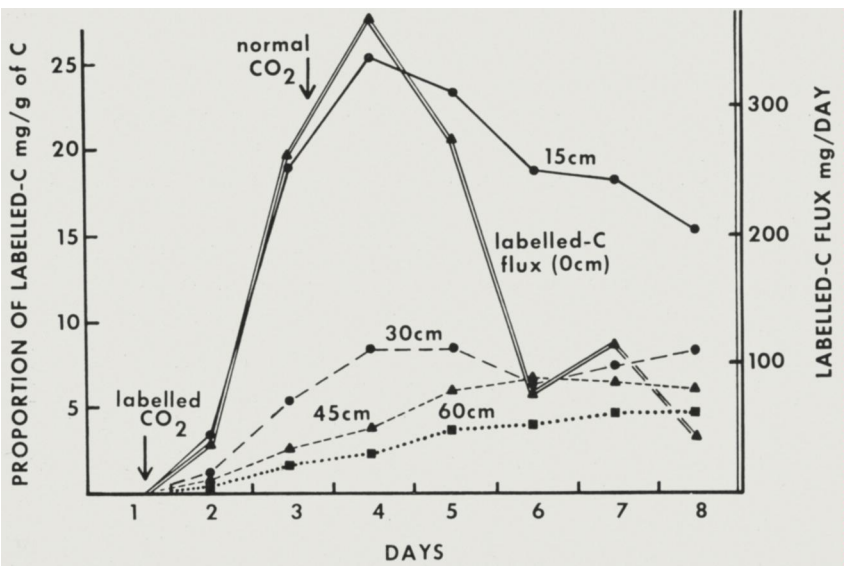

Fig. 4. Proportions of labelled–C in soil air at different depths and daily rates of labelled C evolution at the soil surface (field experiment, August). *Arrows* indicate the time when labelled and unlabelled CO_2 were added.

ty at 36 hours (3300); for the roots of lower depths the specific activity was the highest after 120 hours.

The length of the period of active translocation of labelled compound is illustrated by the proportion of labelled carbon respired by the roots as a function of time (Figure 4). The maximum specific activity measured at 15 cm depth occurs one day after the end of the labelling period. With increasing depth, this maximum is delayed and at 60 cm, 5 days were required to reach maximum labelled carbon respiration.

Root respiration measurement

Knowledge of the radioactivity content (Fig. 4) and concentration of CO_2 (Fig. 5) at various depths in the soil atmosphere made it possible to calculate the flux of labelled carbon at the soil surface (Fig. 4 – double lined curve). The amount of labelled carbon respired by the roots below 30 cm was small and the flux at the surface reached its maximum one day after termination of labelling. The small peak occurring on day 7 of the experiment (Fig. 4) is attributable to increased respiration caused by a small amount of rain which fell on day 6. This was also recorded for total CO_2 concentra-

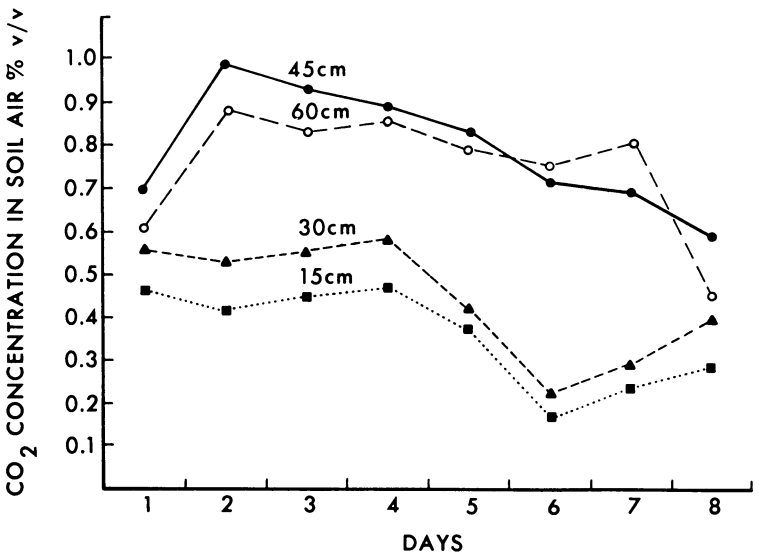

Fig. 5. CO_2 concentration in soil air at various depths (field experiment, August).

tions at 15 and 30 cm depth (Fig. 5). Quantitatively, a total of 1186 mg/m² of labelled carbon had been respired during 8 days out of 4886 mg translocated to the roots after two days labelling.

Labelled carbon distribution

The distribution of biomass and labelled carbon between the different plant parts for two field experiments consisting of 50 hours of labelling followed by 5 days of soil respiration measurements are shown in Table 3. One of the labelling periods was in June, the other

TABLE 3

Biomass–C and labelled carbon distribution in foliage and roots after exposure to $C^{14}O_2$ (field experiment)

		Depth (cm)					
	Shoots	Rhizomes and roots	Roots, depth (cm)				
		0–10	10–25	25–40	40–55	55–70	70–85
June							
Biomass–C, g/m²	89	352	149	85	56	45	35
Labelled–C, mg/m²	2,700	1,500	510	210	34	23	19
Labelled–C, mg/g of C	30.1	4.31	3.41	2.49	0.61	0.51	0.55
Labelled–C distribution (%)	53.6	30.4	10.2	4.2	0.7	0.5	0.4
Soil moisture, pF		3.8	2.2	1.9	1.9	2.1	2.5
August							
Biomass–C, g/m²	94	369	133	92	59	50	49
Labelled–C, mg/m²	3,130	2,180	410	320	220	280	290
Labelled–C, mg/g of C	33.5	5.90	3.08	3.48	3.82	5.55	5.92
Labelled–C distribution (%)	45.9	31.9	6.0	4.7	3.3	4	4.2
Soil moisture, pF		4.61	3.6	2.9	2.7	2.7	2.9

in August. The respiration data (Figs. 4 and 5) shown above were gathered during the August experiment. Comparison with Table 1 indicates the great differences in growth characteristics between the native grass species and wheat. In wheat, 80 to 87 per cent of the label was concentrated above ground. In the native species, approximately 50 per cent was found above ground and 50 per cent in the roots, even though the foliage accounted for only 12 per cent of the total biomass. This indicates a very rapid turnover of the aerial portion of the plants compared to that of the roots. The biomass-

carbon in both parts did not change between June and August indicating that an amount of material equal to that which has been assimilated has disappeared, with a turnover rate of at least one. The biomass of the roots is 8 times larger than that of the shoots, therefore, their turnover must be only 1/8 that of above ground parts since above and beneath ground production is similar (Table 3). If the roots of the virgin grassland turns over every 4 years as Dahlman believes [2], the foliage must turnover at least twice a year.

The distribution of the labelled carbon throughout the root system shows the different growth rates during the two labelling periods. In June the labelled, beneath-ground carbon was concentrated in the 0-10 cm roots and rhizomes and decreased rapidly with depth with as little as 2 per cent found between 55 and 85 cm. The soil moisture data indicate that at this time the soil still had adequate moisture at all depths with a temperature of 12°C at 55 cm. In August, large amounts of carbon were deposited in the 0–10 cm area even though the soil moisture was below the wilting point as usually defined (15 atmosphere retention or pF = 4.2). The relative root growth per unit of biomass in August was as high between 55 and 85 cm as near the surface with a minimum registered between 10 and 55 cm. In June it decreased with depth. This indicates an increase of root biomass at depth during the dry period which can be attributed to the activity of the roots in search of water to supply the all plant system during this period. This agreed with the studies done by Coupland and Johnson [1] who concluded that grassland species show deepest root penetration in the drier climate of the brown soil along the Saskatchewan climatic gradient.

DISCUSSION

The technique for labelling plant material with $C^{14}O_2$ presented in this paper has proven applicable to both laboratory and field studies for determining the distribution of photosynthates within the plant and root and associated microflora respiration. It is relatively inexpensive if regular tracer equipment is available and the technique can be applied to a number of other studies of the carbon cycle in cultivated and virgin systems.

Appreciable radioactivity was recovered in the plant biomass

and the soil atmosphere when the plants were exposed two to three days to concentrations of labelled carbon low enough such that they were not lethal to the plants. In the laboratory, all the $C^{14}O_2$ was recovered and a single exposure to 100 μc/g of carbon was satisfactory for separation of the root and associated microbial respiration and for determining the distribution of carbon within the plant–soil system. Application of such short term labelling has the advantage that during the short period of study, quantitative measurements can be made per unit of time. Care must be taken in inserting the partition between above and beneath ground plant parts such that as little as possible of the shoot bases are included in the beneath ground area.

In the field, and particularly on virgin grassland, due to the density and height of the vegetation, it was found impractical to insert a partition to prevent unlabelled soil CO_2 diluting the added CO_2. This resulted in variability in the specific activity of the CO_2 in the canopy. The determination of the labelled carbon assimilated and respired by the plants and the proportion of the label in each plant or soil compartment was not affected. However, direct measurement of soil and plant respiration was made impossible. In the Matador Project, this was not a major problem for two other measurements of net photosynthesis are available (canopy – infrared CO_2 and microgradient CO_2 techniques). Calculation of the distribution of net photosynthesis values by the use of the C^{14} distribution data will make it possible to differentiate between plant and associated microflora respiration and soil respiration. In a cultivated field where the plants are well spaced, application of a partitition should be possible as it is also on soil cores of native grassland in the growth chamber where smaller areas are involved.

Increasing the size of the canopy beyond that used in this study would result in some air mixing and cooling problems. However, the increased sampling area would be very useful. It would make possible extended sampling of above and beneath ground vegetation with time to enable determination of the turnover rates of the plant and soil materials as the incorporated C^{14} is decomposed.

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