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SITES OF MICROBIAL ASSIMILATION, AND TURNOVER OF SOLUBLE AND PARTICULATE ^{14}C-LABELLED SUBSTRATES DECOMPOSING IN A CLAY SOIL

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Summary—Different types of ^{14}C-labelled substrates, two soluble (glucose and starch) and two particulate (legume and wheat leaves), were incubated in a Vertisol to test the importance of substrate–soil matrix relationships in the processes of soil organic matter decomposition and the location of micro-organisms. Mineralized C (CO_2 ^{12}C, CO_2 ^{14}C) were measured within 66 d of incubation. Sieving and sedimentation procedures were used to fractionate (Light fractions (Lf) >250 μm, Lf 50–250 μm, Heavy fractions (Hf) >50 μm, Hf 2–50 μm, and Hf 0–2 μm) the soil. Biomass C (^{12}C and ^{14}C) in *unfractionated soil* and in fractions was assayed after 3, 38 and 66 d. Comparisons with an unamended soil (control) were made. Decay rates of substrate ^{14}C were highest during the first 3 d of incubation. After 66 d, substrate-derived CO_2 ^{14}C represented 63, 64, 59 and 51%, of input ^{14}C in soils amended with the glucose, starch, legume and wheat, respectively. Unlike ^{14}C, rates of mineralization of ^{12}C in amended and unamended soils remained more uniform throughout. Total biomass C in soluble substrate-amended soils was similar to that in the control, despite about 60% of total biomass C being derived from ^{14}C substrate amendments. By contrast, decomposition of particulate substrates increased total biomass C concentration at day 3. There was little or no turnover of ^{14}C apparent within the first 3 d, as indicated by high (0.60) growth efficiencies (biomass ^{14}C/[biomass ^{14}C + CO_2 ^{14}C]). Fraction weights were constant. Irrespective of treatments, the silt-size fraction (Hf 2–50 μm) was the most abundant (about 51% of total soil weight). This fraction concentrated 65% of the clay fraction as microaggregates. The fraction (Hf > 50 μm) approximated sand particles (> 50 μm). After 3 d, for soils amended with soluble substrate, most (about 65%) of the recovered biomass ^{14}C was associated with the silt-size fraction (Hf 2–50 μm) and accounted for 79 and 63% of the total biomass C of that fraction in the glucose- and starch-amended soils, respectively. For soils amended with particulate residues, biomass ^{14}C was bimodally distributed, with peak amounts in the silt-size fraction (Hf 2–50 μm) and the light fraction >250 μm (Lf >250 μm). In these latter treatments the substrate-derived biomass ^{14}C associated with the fraction Lf >250 μm corresponded broadly to the enhanced total biomass C of the unfractionated soil, when compared with that of the control. Irrespective of substrate amendments, biomass ^{14}C located in the light fraction (Lf >250 μm) had disappeared by 66 d. This decline accounted for more than 50% of biomass ^{14}C decline from unfractionated soil in particulate plant residue-amended soils. In contrast, in soils amended with soluble substrates, most of the decline in unfractionated soil originated in the silt-size fraction (Hf 2–50 μm). The nature of the substrate amendment ensured different sites of microbial activity and turnover, amended particulate residues offering new sites for micro-organisms and soluble compounds stimulating those micro-organisms located within soil matrix (microaggregates 2–50 μm). © 1997 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

The extent to which organic compounds are decomposed in soils depends on substrate quality and soil characteristics. The quality of plant residues can be assessed in many ways, e.g. C-to-N ratio, lignin content, and, in combination, allowed prediction of the rates of residue decomposition (Herman *et al.*, 1977). The proportions of plant residue C or N in soluble components have also been proposed as an accurate index of the extent of early decomposition (Iritani and Arnold, 1960; Reinertsen *et al.*, 1984), although the relationship is improved if the plant residue pool encompasses cold-water soluble C and a particulate C pool of intermediate availability.

Many authors have examined the effects of soil structure on the distribution and activities of the soil biota, including work on the distribution of soil micro-organisms in particle-size fractions (Elliott, 1986; Gupta and Germida, 1988; Hattori, 1988; Kabir *et al.*, 1994) and soil porosity (Killham *et al.*, 1993). Amato and Ladd (1992) obtained a positive correlation between glucose-derived or plant residue-derived biomass C and the clay content of 23 Australian soils (pH >7.0). Amato and Ladd (1992) and Ladd *et al.* (1992) indicated that soil microporosity *per se* did not explain differences in biomass ^{14}C turnover between soils. Biomass ^{14}C

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Table 1. Fractionation of legume and wheat leaves

Fraction	Legume leaves		Wheat leaves	
	Weight (mg C g^{-1} plant)	Specific activity (MBq g^{-1} C)	Weight (mg C g^{-1} plant)	Specific activity (MBq g^{-1} C)
Whole material	390	2.994	380	2.741
Soluble fractions				
Cold water	66	3.203	66	2.913
Hot water	16	3.116	19	2.360
Σ	82	3.186	85	2.788
Insoluble fraction	308	2.940	295	2.727

stability was greater in a Vertisol than in an Alfisol, although the volume of pores of neck dia. >6 μm, in which most of the biomass ^{14}C was expected to reside, was similar in both soils. They suggested that the volume of water-filled pore space could be of higher importance in biomass turnover.

The enhanced mineralization of endogenous organic matter following the addition of readily-decomposable substrates to soils has been thoroughly studied (Löhnis, 1926; Broadbent, 1947; Broadbent and Bartholomew, 1948), although the origins of this extra C mineralized from soil organic pools, have not yet been evaluated. Nevertheless, evidence suggests that this primed C is derived from the turnover of dead cells by secondary populations (Dalenberg and Jager, 1989; Mary *et al.*, 1993).

Simulation models of C and N behaviour in soil involve a description of C and N flux through the soil microbial biomass (Jenkinson and Rayner, 1977; Paul and van Veen, 1978; van Veen *et al.*, 1987; McGill *et al.*, 1981; Parton *et al.*, 1988; Ladd *et al.*, 1995a). In relation to these models, several physical and chemical procedures have been proposed to describe the nonmicrobial compartments. However, soil physical fractionations have yielded better fits between predicted and experimental measurements during the past 20 yr (Christensen, 1985; Balesdent *et al.*, 1988; Cambardella and Elliott, 1992). Most of these methods were directed at separating fractions with respect to their size and density. However, very little of the extensive literature on physical fractionation is aimed at describing soil biomass turnover associated with soil fractions.

We investigated the sites of microbial assimilation and turnover of ^{14}C-labelled soluble and particulate plant residues incorporated in a Vertisol. This highly aggregated soil provided us with a good model to test the importance of substrate–soil matrix relationships in the processes of soil organic matter decomposition and the location of soil micro-organisms. We hypothesized that clay surfaces would affect the behaviour of soluble substrate C more extensively than that of particulate plant residue C and that the nature of the substrate amendments would modify the biomass location and turnover.

MATERIALS AND METHODS

Soil, substrates and incubation conditions

Soil was sampled to 10 cm depth from a Vertisol (Black Earth) at a site near Northfield, South Australia in late Spring, 1993. Pending further use, the soil was stored at 4°C at its field moisture content, which was equivalent to 20% of its water holding capacity (WHC). Contents (w/w) of organic C, clay (<2 μm dia.) and sand (>50 μm dia.) components were 1.9, 50, and 22% respectively.

The substrates used were ^{14}C-labelled glucose (2.769 MBq g^{-1} C), starch (1.364 MBq g^{-1} C), legume leaves (2.994 MBq g^{-1} C), and wheat leaves (2.741 MBq g^{-1} C). The proportions of C and ^{14}C of legume and wheat leaves that were soluble in cold and hot water were determined by a method adapted from Waksman and Stevens (1928), and are shown in Table 1. Briefly, 500 mg of dry plant residue was shaken for 1 h in 10 ml of cold water, filtered and then washed with 10 ml of water (cold water-soluble C). The washed residues were resuspended in 10 ml of water, placed in boiling water for 1 h without shaking, and similarly filtered and washed (hot water-soluble C). The cold and hot water filtrates were analysed for total C and ^{14}C. The results in Table 1 show that the specific activities (MBq g^{-1} C) of cold and hot water-soluble C, and of the insoluble C were approximately similar for a given plant material, indicative of a near homogeneous labelling of the legume and wheat leaves.

The soil was thoroughly mixed and sieved (8 mm), its water content adjusted to 40% WHC and conditioned for 2 wk at 28°C. Pores of equivalent neck dia. <3 μm were calculated to be filled with water during the conditioning (Killham *et al.*, 1993). Subsamples of soil (equivalent to 50 g oven dried-soil) were incubated in triplicate with substrates, added to a final concentration of 1 mg C g^{-1} soil. Glucose and starch were added as solution to the soil, whereas legume and wheat leaves were added as oven-dried (60°C, overnight) segments unground but cut (<500 μm). An unamended soil was included as a control. Soil bulk density was adjusted to 1.0. Soil moisture content was adjusted to 60% WHC by the addition of substrate solution or water, thus filling pores of an equivalent dia. calculated to be <30 μm. The incubation lasted for up

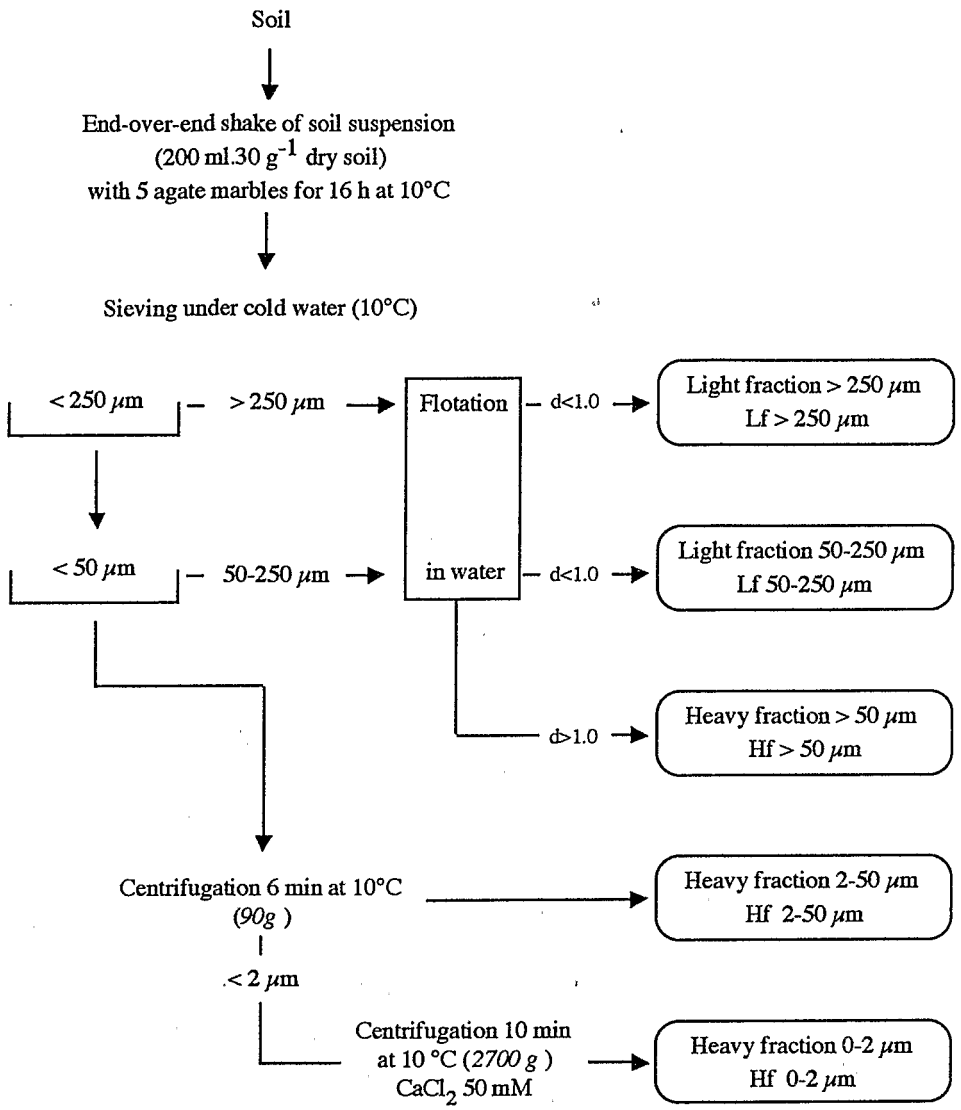

Fig. 1. Procedure for soil fractionation.

to 66 d at 25°C, under conditions as described by Ladd *et al.* (1992).

Soil fractionation

After incubation for 3, 38 and 66 d, soils were partially dispersed by the Bruckert (1979) procedure, as follows: for each substrate treatment and sampling time, a subsample (equivalent to 10 g oven dried soil), was taken from each of the triplicate incubation vessels. The subsamples were pooled and suspensions of the soil [30 g in 200 ml of cold, (< 10°C), distilled water] were rolled for 16 h with five agate marbles, in order to disrupt macroaggregates (> 250 μm dia), and to mainly preserve microaggregates (< 50 μm dia) (Jocteur Monrozier *et al.*, 1991) (Fig. 1).

Each soil suspension was successively sieved to yield coarse and fine sand-size fractions, of dia. > 250 μm and 50–250 μm, respectively. Light fractions (Lf) were separated from the coarse sand- and fine sand-size ranges by flotation in water. The heavy sand-size fractions (> 250 μm and 50–250 μm) were pooled and mixed to give a single sand-size fraction (Hf > 50 μm). Particles 2–50 μm and < 2 μm dia. were separated by centrifugation at 90 g for 6 min. Fractions in the silt-size (Hf 2–50 μm) and clay-size (Hf < 2 μm) ranges were flocculated from suspension with $CaCl_2$ (10 mM, final concentration) and sedimented by centrifugation at 2500 g for 10 min. The fractionation schedule required 5 d for a complete set of five samples.

Analyses

CO_2 C and CO_2 ^{14}C Decomposition of added substrates and turnover products was determined from measurements of CO_2 C and CO_2 ^{14}C from

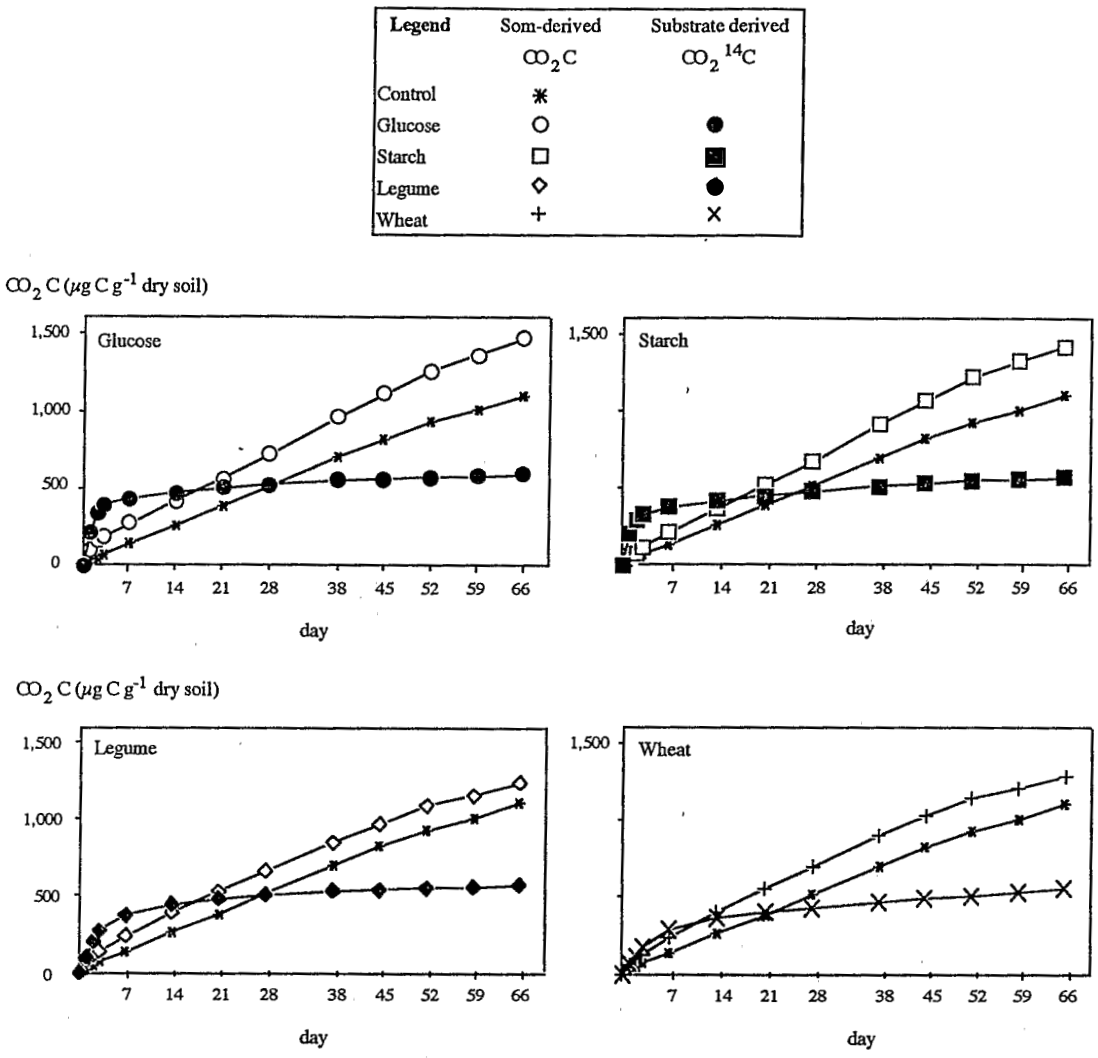

Fig. 2. Evolution of CO_2 C from Som and added substrate in glucose-, starch-, legume- and wheat-amended soils.

each vessel (three replicates). Total CO_2 absorbed in NaOH solution was analysed by titration of aliquots (2 ml) with standard HCl. Absorbed CO_2 ^{14}C was measured using 0.5 ml aliquots of NaOH solution, mixed with 10 ml of LKB Hiphase III scintillation cocktail. Analyses of evolved CO_2 ^{14}C and soil organic ^{14}C demonstrated a ^{14}C deficit on incubation. After 3 d, following maximal rates of CO_2 ^{14}C evolution from all substrates, the magnitude of the ^{14}C deficit was directly related to the amount of substrate ^{14}C converted to CO_2 ^{14}C and trapped in NaOH solution (glucose > starch > legume > wheat). Because soil inorganic ^{14}C (carbonate) was found to be very low (< 0.03% of input ^{14}C) in each treatment, the ^{14}C deficit may have resulted from the failure to trap all CO_2 ^{14}C produced during the first few days of rapid microbial decomposition of added susbrates, i.e. nonabsorbed CO_2 ^{14}C may have been lost when NaOH solution traps were removed and renewed. To test this possibility, replicated soil samples were amended with ^{14}C-glucose and incubated for 24 h under the same experimental conditions. Within this period, NaOH traps were either changed four times (after 1, 4, 8, and 24 h) or were changed only once (after 24 h). The quantities of CO_2 ^{12}C and CO_2 ^{14}C measured in triplicate after 24 h were lower when NaOH traps were sampled four times than when traps were sampled once only: trapped CO_2 ^{14}C accounted for 8.5 and 12% of input ^{14}C, respectively. The results also showed that trapping of CO_2 ^{12}C was little affected by the frequency of change of NaOH solutions. We can therefore assume that the low ^{14}C recoveries were due to CO_2 ^{14}C losses occuring during the first 3 d of incubation as NaOH traps were changed. Therefore, measured values were adjusted accordingly.

Table 2. Residual organic ^{14}C in soil and evolved CO_2 ^{14}C after incubation of isotope-labelled substrates

Incubation time (d)	Substrate	Organic ^{14}C (% of input ^{14}C)	CO_2 ^{14}C (% of input ^{14}C)	CO_2 ^{14}C (% of CO_2 total C)
3	glucose	58 (c)	42 (a)	68
	starch	63 (c)	37 (a)	74
	legume	73 (b)	27 (b)	66
	wheat	84 (a)	16 (c)	57
38	glucose	39 (c)	61 (a)	37
	starch	41 (c)	59 (a)	36
	legume	49 (b)	51 (b)	38
	wheat	58 (a)	42 (c)	34
66	glucose	37 (b)	63 (a)	28
	starch	36 (b)	64 (a)	29
	legume	41 (b)	59 (a)	32
	wheat	49 (a)	51 (a)	30

At each sampling date, a different letter indicated a significative difference (PLSD Fisher, $p < 0.05$).

Biomass C and biomass ^{14}C The amounts of total microbial biomass C and substrate-derived biomass ^{14}C were determined in triplicate in moist unfractionated soils sampled after 3, 38 and 66 d of incubation, and in duplicate in fractions prepared from them. Free water was removed from slurries of fractions by centrifugation, prior to biomass assay. Biomass C and ^{14}C of moist unfractionated soil and moist particle fractions were estimated by fumigation–extraction methods (Amato and Ladd, 1988; Ladd and Amato, 1988). Total biomass C of unfractionated soil and soil fractions was estimated from the gain in ninhydrin-reactive N after fumigation, multiplied by 21 (Amato and Ladd, 1988). Biomass ^{14}C of unfractionated soil and soil fractions was determined from the gain of extractable ^{14}C after fumigation, multiplied by a slightly lower factor, 3.05 for unfractionated soil and 2.65 for soil fractions, than that (3.25) proposed by Ladd and Amato (1988), to take into account the effect of a higher soil moisture content on assay performance (Chotte, unpublished data).

Residual organic ^{14}C Duplicate subsamples of soil and soil fractions were oven-dried (80°C) and ground by hand *in toto*. Residual organic ^{14}C was determined on subsamples of these materials by wet combustion (Amato, 1983).

RESULTS

C mineralization

Irrespective of the substrate, the rate of mineralization of substrate-derived ^{14}C was highest during the first 3 d of incubation, and slowed markedly thereafter (Fig. 2, Table 2). As a percentage of input ^{14}C, CO_2 ^{14}C after 3 d, ranged from 42% in the glucose-amended soil, 37% in the starch-amended soil and 27% in the legume-amended soil, to 16% in the wheat-amended soil. At the end of the incubation (66 d), the substrate-derived CO_2 ^{14}C ranged from 64 to 51% of the input ^{14}C (Table 2). Based on measured values of residual organic ^{14}C, net average decay rates of residual organic ^{14}C between incubation times, 3–66 d, were $7.3\ 10^{-3}\ d^{-1}$ for glucose-amended soil, $9.1\ 10^{-3}\ d^{-1}$ for the starch- and legume-amended soils, and $8.5\ 10^{-3}\ d^{-1}$ for the wheat-amended soils.

Table 3. Som-derived CO_2 C evolved from soils incubated with ^{14}C-labelled substrates

Substrate	Incubation time (d)	Som-derived CO_2 C ($\mu g\ C\ g^{-1}$ soil) Mean	S.D.	(% of control)
control	0–3	65	5.2	1.00
	3–38	634	57.6	1.00
	38–66	398	27.9	1.00
	0–66	1097	98.0	1.00
glucose	0–3	180	10.8	2.77
	3–38	780	62.4	1.23
	38–66	510	45.9	1.28
	0–66	1470	88.2	1.34
starch	0–3	100	7.5	1.54
	3–38	810	64.8	1.28
	38–66	510	44.9	1.28
	0–66	1420	125.0	1.29
legume	0–3	140	9.1	2.15
	3–38	720	57.6	1.14
	38–66	390	31.2	0.98
	0–66	1250	100.0	1.14
wheat	0–3	130	11.7	2.00
	3–38	770	76.1	1.21
	38–66	400	32.1	1.01
	0–66	1300	117.2	1.19

Substrate-derived CO_2 ^{14}C represented from 57 to 68% of the total CO_2 C evolved after 3 d and 28–32% of total CO_2 C evolved after 66 d (Table 2). Thus, unlike ^{14}C, rates of mineralization of ^{12}C from soil organic matter (Som), both in amended and unamended soils, were relatively uniform throughout the incubation (Fig. 2).

The total amounts of ^{12}C evolved were higher in the amended soils than in the control. After 66 d, Som-derived CO_2 ^{12}C amounted to 1470, 1420, 1250 and 1300 $\mu g\ C\ g^{-1}$ soil from the glucose-, starch-, legume-, and wheat-amended soils respectively, compared with 1097 $\mu g\ C\ g^{-1}$ soil from the unamended control (Table 3). The enhanced mineralization of Som-derived C from substrate-amended soils was particularly evident during the 0–3 d period, when the Som-derived CO_2 C was, respectively, 2.70, 1.54, 2.15 and 2.00 times higher from the glucose-, starch-, legume-, and wheat-amended soils than that from the control. This effect weakened after 3 d. Nevertheless, a greater rate of evolution of CO_2 ^{12}C from the glucose- and starch-amended soils, compared with the control, continued throughout the incubation. The stimulation of CO_2 ^{12}C from the plant residue-amended soils was not apparent during the final period (38–66 d) of incubation.

Biomass ^{14}C and C in unfractionated soils

After 3 d, substrate-derived biomass C in the starch-, glucose-, legume-, and wheat-amended soils amounted to 610, 550, 350 and 290 $\mu g\ C\ g^{-1}$ soil,

Table 4. Biomass C, (total, Som- and substrate-derived), in soils incubated with ^{14}C-labelled substrates

Substrate	Incubation time (d)	Total biomass C ($\mu g\ C\ g^{-1}$ soil)	Som-derived biomass ^{12}C ($\mu g\ C\ g^{-1}$ soil)	Substrate-derived biomass ^{14}C		
				($\mu g\ C g^{-1}$ soil)	(% of organic ^{14}C)	(/(Biomass $^{14}C + CO_2\ ^{14}C$)
control	3	1050	1050	0	n.a.	n.a.
	38	1060	1060	0	n.a.	n.a.
	66	930	930	0	n.a.	n.a.
glucose	3	1020	470	550	102	0.58
	38	1090	740	350	97	0.64
	66	930	680	250	74	0.30
starch	3	1050	440	610	107	0.64
	38	1080	850	230	62	0.44
	66	960	790	170	53	0.23
legume	3	1300	950	350	48	0.56
	38	1110	960	150	31	0.28
	66	940	810	130	32	0.18
wheat	3	1190	900	290	33	0.63
	38	1070	930	140	23	0.30
	66	960	850	110	21	0.19

and biomass ^{14}C accounted for respectively 68, 59, 35 and 27% of input substrate ^{14}C. At the end of the incubation, biomass ^{14}C represented 19, 27, 12 and 10% of the input ^{14}C in the starch-, glucose-, legume- and wheat-amended soils, respectively.

Between days 3 and 38, biomass ^{14}C concentrations decreased in all treatments. Net first order decay rates of substrate-derived biomass ^{14}C during this period were 0.028, 0.013, 0.024 and 0.021 d^{-1} in the starch-, glucose-, legume-, and wheat-amended soils, respectively. During the period 38–66 d, net decay rates (d^{-1}) of biomass ^{14}C slowed further (0.011, 0.012, 0.008, 0.009, respectively).

The percentages of organic residual ^{14}C as biomass ^{14}C in the glucose- and starch-amended soils were higher than 100% at day 3 (Table 4). At this sampling time, the percentages were 48% in the legume-amended soil and 33% in the wheat-amended soil. The ratio, biomass ^{14}C-to-biomass ^{14}C plus $CO_2\ ^{14}C$, an expression of the efficiency of substrate utilization for microbial growth, was close to 0.60 in each treatment. This ratio decreased with incubation time. At 66 d, they were higher in the glucose- (0.30) and starch- (0.23) amended soils than in the legume- (0.18) and wheat- (0.19) amended soils. The decrease in this ratio was therefore higher in the particulate plant residue-amended soils than in the soluble substrate-amended soils. The ratio, biomass ^{14}C-to-$CO_2\ ^{14}C$ decreased from 1.41, 1.85, 1.30 and 1.71 at day 3 to 0.42, 0.30, 0.21 and 0.20 at day 66 in the glucose-, starch-, legume- and wheat-amended soils, respectively.

Table 5. Recoveries after soil fractionation of soil weight, organic (substrate-derived) and biomass C (substrate- and Som-derived)

Substrate	Recovery after fractionation*			
	Weight	Organic ^{14}C	Total biomass C	Substrate-derived biomass ^{14}C
control	0.98	n.a.	1.00	n.a.
glucose	1.00	0.96	1.00	1.04
starch	1.00	0.97	1.03	0.98
legume	0.99	0.97	1.00	1.03
wheat	0.98	0.96	1.01	1.01

* [(Σ fraction/unfractionated soil) × 100], (average for the three sampling dates).
n.a.: not applicable.

After 3 d, total biomass C in the control soil (1050 $\mu g\ C\ g^{-1}$ soil) was the same as that of the glucose- and the starch-amended soils. By contrast, soils with particulate plant residue amendments yielded the highest biomass C (1300 and 1190 $\mu g\ C\ g^{-1}$ soil in the legume- and wheat-amended soils, respectively) (Table 4). Total biomass C increased slightly in the control and in the glucose- and starch-amended soils during the 3–38 d period, then declined. At the end (66 d) of incubation, the total biomass C for unamended and all amended soils were of similar concentrations (about 940 $\mu g\ C\ g^{-1}$ soil).

Biomass ^{14}C (substrate-derived), as a percentage of total biomass C, was higher in soils amended with the soluble substrates than those with the plant residues. At 3 d, values ranged from 58% in the starch-amended soil and 54% in the glucose-amended soil to 27% and 24% in the legume- and wheat-amended soils, respectively. By the end of the incubation, the proportions were 2–3 times lower than that at 3 d in each treatment.

Som-derived biomass ^{12}C, calculated by the difference between total and substrate-derived biomass C was, at 3 d, higher in the legume- (950 $\mu g\ C\ g^{-1}$ soil) and the wheat-amended soil (900 $\mu g\ C\ g^{-1}$ soil) than in the glucose- (470 $\mu g\ C\ g^{-1}$ soil) and the starch-amended soil (440 $\mu g\ C\ g^{-1}$ soil) (Table 4). Thereafter, between days 3 and 38, Som-derived biomass ^{12}C increased in the soils amended with glucose and starch, and remained essentially constant in the control soil, and the soils with plant residue amendments. Minor decreases in biomass ^{12}C were observed in all soils on further incubation. At all times, concentrations of biomass ^{12}C were higher in the control than in the amended soils and

Table 6. Concentration of biomass C, Som-derived and substrate-derived, in fractions of soils incubated for 3 d

	Som-derived biomass ^{12}C					Substrate-derived biomass ^{14}C				
	Lf > 250 μm	Lf > 50–250 μm	Hf > 50 μm	Hf 2–50 μm	Hf 0–2 μm	Lf > 250 μm	Lf 50–250 μm	Hf > 50 μm	Hf 2–50 μm	Hf 0–2 μm
Substrate	(mg C g^{-1} fraction)									
control	7.00	2.14	0.02	1.37	0.92	0	0	0	0	0
glucose	4.69	0.91	0	0.27	0.69	4.97	0.99	0.01	0.80	0.50
starch	5.85	1.19	0.05	0.51	0.18	1.54	0.59	0	0.73	0.69
legume	4.97	1.60	0.03	1.17	0.77	17.45	0.39	0	0.23	0.31
wheat	7.52	1.86	0.05	0.90	0.98	13.92	0.33	0	0.24	0.26

were higher in soils with particulate substrates than in those with soluble amendments.

Fractionation of soils

Soil weight distributions in fractions No loss of total soil weight occured on soil fractionation. Recoveries of substrate-derived organic ^{14}C and of biomass ^{14}C were about 100% in all treatments, except for the glucose- and legume-amended soils after 3 d of incubation, when recoveries of biomass ^{14}C were excessively high (113 and 108% respectively,). Values shown in Table 5 are averages for the three sampling times.

There was no significant effect of substrate amendment on fraction weight distribution throughout the incubation. The silt-size fraction (Hf 2–50 μm) constituted the major fraction and represented 50.8% of total soil weight, on average. Sand-size fractions (Hf > 50 μm) accounted for 21% of the soil weight, thus approximating the proportion of soil accounted for by the sand particles (> 50 μm) when determined by conventional particle size analysis. Clay-size fractions, dispersed by Bruckert's method, amounted to 17.8% of total soil weight, compared with 50% by conventional particle size analysis. Thus, 35% of the clays of the Northfield soil were dispersed by our fractionation procedure, 65% remaining as clay-aggregates in the fraction comprised of silt-size particles.

Concentrations of substrate-derived and Som-derived biomass C in fractions

Patterns of the concentration of substrate-derived biomass C and Som-derived biomass C were broadly similar in all treatments (Table 6). After 3 d, they peaked in the Lf > 250 μm fraction. Concentrations of substrate-derived biomass C in this fraction from soils amended with starch, glucose and particulate plant residues were, respectively, lower, slightly higher and much higher than Som-derived biomass C. Concentrations of Som-derived biomass C in this fraction from amended soils were lower than that of the fraction from unamended soil, except from the wheat-amended soil. For the fraction (Lf 50–250 μm) concentrations of substrate-derived biomass C in glucose-amended soil was slightly higher than Som-derived biomass C, but for the other amended soils, the reverse was true. For all amended soils, Som-derived biomass C in the fraction (Lf 50–250 μm) was lower than that in the fraction from control soil.

Substrate-derived biomass C in the silt-size fraction (Hf 2–50 μm) from the glucose- and starch-amended soils was present in higher concentrations than Som-derived biomass C. By contrast, concentrations of substrate-derived biomass C in this fraction from soil amended with particulate plant residues were lower than those of Som-derived biomass C of the fraction. Concentrations of Som-derived biomass C in the silt-size fraction (Hf 2–50 μm) from the amended soils were lower than that of the fraction from the unamended soil. Except for the glucose-amended soil, the concentration of substrate-derived biomass C in the clay-size fraction (Hf 0–2 μm) from amended soils was broadly equivalent to that of the silt-size fraction. These amounts in the Hf 0–2 μm were respectively lower and higher than Som-derived biomass C in the fraction from starch-amended soil and glucose-, legume- and wheat-amended soils.

Irrespective of treatment, the sand-size fraction (Hf > 50 μm) contained essentially neither substrate-derived biomass C nor Som-derived biomass C.

Distribution of total biomass C, Som-derived biomass ^{12}C, and substrate-derived biomass ^{14}C in soil fractions

Fraction Lf > 250 μm Total biomass C was recovered in higher proportions in Lf > 250 μm in amended soils than in the control, and particularly in soils amended with particulate substrates. Som-derived biomass ^{12}C in this fraction from the control soil was in very similar proportions to those of the fraction from the amended soils, except in the starch-amended soil. The distribution of substrate-derived biomass ^{14}C in soil fractions differed according to treatments. After 3 d, biomass ^{14}C associated with the Lf > 250 μm amounted only to 30 μg ^{14}C g^{-1} soil in the glucose- and starch-amended soils (Table 7). These quantities were far lower than those in the legume (150 μg ^{14}C g^{-1} soil)

Table 7. Biomass C (Som-and substrate-derived) in fractions of soils incubated with ^{14}C-labelled substrates

Substrate	Time (d)	Som-derived biomass ^{12}C				Substrate-derived biomass ^{14}C									
		Lf >250 μm	Lf 50–250 μm	Hf >50 μm	Hf 2–50 μm	Hf 0–2 μm	Lf 250 μm	Lf 50–250 μm	Hf >50 μm	Hf 2–50 μm	Hf 0–2 μm	Lf >250 μm	Lf 50–250 μm	Hf >50 μm	Hf 2–50 μm	Hf 0–2 μm
		(μg C g^{-1} soil)					(μg C g^{-1} soil)					(% of Total biomass C)				
control	3	40	170	0	670	170	0	0	0	0	0	n.a.	n.a.	n.a.	n.a.	n.a.
	38	40	170	0	650	200	0	0	0	0	0	n.a.	n.a.	n.a.	n.a.	n.a.
	66	10	150	0	570	200	0	0	0	0	0	n.a.	n.a.	n.a.	n.a.	n.a.
glucose	3	50	100	0	190	130	30	70	0	370	80	38	41	n.a.	66	38
	38	40	120	10	390	180	10	40	0	270	30	20	25	n.a.	41	14
	66	40	130	0	320	190	0	40	0	170	30	n.a.	24	n.a.	35	14
starch	3	90	90	10	230	20	30	50	0	400	130	25	36	n.a.	63	87
	38	60	130	10	490	160	10	20	0	160	40	14	13	n.a.	25	20
	66	50	130	10	410	190	0	20	0	110	40	n.a.	13	n.a.	21	17
legume	3	60	170	0	580	140	150	40	0	110	50	71	19	n.a.	16	26
	38	50	160	10	600	140	10	20	0	100	20	17	11	n.a.	14	13
	66	40	160	10	460	140	0	20	0	80	30	n.a.	11	n.a.	15	18
wheat	3	50	210	10	450	180	100	30	0	110	50	67	13	n.a.	20	22
	38	60	130	10	550	180	20	10	0	90	20	25	7	n.a.	14	10
	66	30	150	10	460	200	10	30	0	60	20	25	12	n.a.	12	9

n.a.: not applicable.

and wheat-amended soils (100 μg ^{14}C g^{-1} soil). Biomass ^{14}C, as a percentage of total biomass C in this fraction, peaked at 71% in the legume-amended soils and decreased to 67, 38 and 25% in the wheat-, glucose- and starch-amended soils, respectively.

On further incubation, both the proportions and amounts of recovered biomass ^{14}C in the fraction Lf >250 μm declined markedly in each treatment, especially between 3 and 38 d (Table 8). Biomass ^{14}C disappeared entirely in this fraction from the glucose-, starch- and legume-amended soils at the end of the incubation. For this fraction from the wheat-amended soil, the decrease in biomass ^{14}C was 90%, when compared with the amount present at day 3. The net decrease of biomass ^{14}C in the fraction Lf >250 μm between days 3 and 66 accounted for 68% of the total net decline of biomass ^{14}C in the legume-amended soil, for 50% in the wheat-amended soil, but only 10 and 7% in the glucose- and starch-amended soils, respectively (Table 8). Som-derived biomass ^{12}C of the fraction Lf >250 μm declined less extensively than did biomass ^{14}C and, by the end of the incubation, was in higher amounts in the amended soils than in the control (Table 7).

Fraction Lf 50–250 μm At 3 d, total biomass C in the fraction Lf 50–250 μm was lower in the soil amended with starch, similar in that amended with glucose and higher in those amended with legume and wheat than biomass C in the fraction from unamended control soil. Substrate-derived biomass ^{14}C in the fraction Lf 50–250 μm accounted for 70 μg C g^{-1} soil in the glucose-amended soil; amounts were less (50, 40 and 30 μg C g^{-1} soil, respectively) in the starch-, legume- and wheat-amended soils. These amounts represented similar proportions of recovered biomass ^{14}C in each of the treatments. By contrast, at day 3, the amounts of Som-derived biomass ^{12}C recoverable in the Lf 50–250 μm fraction, were higher in the legume- and wheat-amended soils than in this fraction from soils incubated with glucose and starch. Biomass ^{14}C in this fraction, as a percentage of total biomass C, peaked at 41% in the glucose-amended soil, and at 36, 19 and 13% in the starch-, legume-, and wheat-amended soils, respectively. On continued incubation, substrate-derived biomass ^{14}C of the fraction Lf 50–250 μm declined disproportionately in all treatments, such that at the end of the incubation, it represented 24, 13, 12 and 11% of total biomass C of the fraction in the glucose-, starch-, wheat- and legume-amended soils, respectively.

Fraction Hf 2–50 μm At 3 d, substrate-derived biomass ^{14}C in the fraction Hf 2–50 μm, accounted for the highest proportions of recovered biomass ^{14}C in the glucose- (67%) and starch-amended (66%) soils. For the legume and wheat amendments, biomass ^{14}C in this silt-size fraction corre-

Table 8. Decline of biomass ^{14}C in fractions of soils incubated with ^{14}C-labelled substrates during the period 3–66 d

	(% of biomass ^{14}C of fraction at day 3)					(% of total decline of biomass ^{14}C soil)				
Substrate	Lf >250 μm	Lf 50–250 μm	Hf >50 μm	Hf 2–50 μm	Hf 0–2 μm	Lf >250 μm	Lf 50–250 μm	Hf >50 μm	Hf 2–50 μm	Hf 0–2 μm
glucose	100	43	n.a.	54	62	10	10	n.a.	65	15
starch	100	60	n.a.	72	69	7	7	n.a.	66	20
legume	100	50	n.a.	27	40	68	9	n.a.	14	9
wheat	90	33	n.a.	54	60	50	5	n.a.	28	17

sponded, respectively, to 32 and 38% of recovered biomass ^{14}C. Som-derived biomass ^{12}C in the fraction Hf 2–50 μm from the amended soils was lower than that from the control. The differences were particularly marked in the fraction from the glucose- and starch-amended soils where biomass ^{12}C represented only about 1/3 of that measured in Hf 2–50 μm from the control. Thus, substrate-derived biomass ^{14}C accounted for more than 60% of the total biomass C of the silt-size fraction from the soils incubated with glucose and starch substrates, whereas most of the total biomass located in this fraction from the legume- and wheat-amended soils was derived from soil organic matter metabolism (Table 7).

Substrate-derived biomass ^{14}C located in the fraction Hf 2–50 μm, declined in all treatments on further incubation. As a percentage of biomass ^{14}C present at 3 d in this fraction, the declines were highest (72% and 54%) in the starch- and glucose-amended soils, respectively (Table 8). The magnitudes of the decline of substrate-derived biomass ^{14}C in this silt-size fraction Hf 2–50 μm accounted for most of the total decline of biomass ^{14}C in whole soils amended with starch (66%) and glucose (65%), but for a minority of the total decline of biomass ^{14}C of whole soils amended with wheat (28%) and legume (14%). During the period 3–38 d, Som-derived biomass ^{12}C of the silt-size fraction increased from 190 μg C g^{-1} soil to 390 μg C g^{-1} soil in the glucose-amended soil and from 230 μg C g^{-1} soil to 490 μg C g^{-1} soil in the starch-amended soil. Changes were relatively minor for the control and plant residue-amended soils. Biomass ^{12}C declined slightly in all treatments during the 38–66 d period. The amounts of Som-derived biomass ^{12}C were higher in the control than in amended soils throughout the incubation. Substrate-derived biomass ^{14}C accounted for 15% of total biomass C located in the Hf 2–50 μm fraction in the legume-amended soil at 66 d, and 21, 35 and 12% in the starch-, glucose-, and wheat-amended soils, respectively.

Fraction Hf 0–2 μm After 3 d, substrate-derived biomass ^{14}C associated with the clay-size fraction, Hf 0–2 μm, represented broadly similar proportions of total biomass ^{14}C recovered in amended soils. As percentages of total biomass C of the fraction, biomass ^{14}C ranged from 87%, in the starch-amended soil, to 38, 26, and 22% in the glucose-, legume-, and wheat-amended soils, respectively. On continued incubation, these proportions declined in each treatment due principally to decreases of biomass ^{14}C concentrations.

Distribution of non-biomass ^{14}C in soil fractions

Non-biomass ^{14}C was calculated as the difference between total organic ^{14}C and biomass ^{14}C in respective whole soils and soil fractions (Table 9). After 3 d of incubation, no non-biomass ^{14}C was determined to be present in the glucose- and starch-amended soils and in fractions. Non-biomass ^{14}C was calculated to be present in the glucose-amended soil only by day 66, and in the starch-amended soil

Table 9. Formation of non-biomass ^{14}C during the incubation of soils with ^{14}C-labelled substrates

			Fractions				
		Unfractionated soil	Lf >250 μm	Lf 50–250 μm	Hf >50 μm	Hf 2–50 μm	Hf 0–2 μm
	Time (d)		(μg C g^{-1} soil)				
glucose	3	0	0	0	0	0	0
	38	0	0	0	0	0	0
	66	80	10	10	0	50	10
starch	3	0	0	0	0	0	0
	38	110	0	10	0	90	20
	66	140	10	10	0	90	30
legume	3	360	150	90	0	80	40
	38	330	90	40	0	160	40
	66	270	30	50	0	140	50
wheat	3	560	380	50	0	100	30
	38	430	260	40	0	90	40
	66	390	140	40	0	150	60

by day 38, increasing by day 66. The increases were principally due to non-biomass ^{14}C associated with the silt-size fraction. By contrast, non-biomass ^{14}C, by day 3, represented 38 and 57% of input ^{14}C in the legume- and wheat-amended whole soils, respectively. Much of the non-biomass ^{14}C in these soils at this time was associated with the fraction Lf $>250\ \mu m$ (42 and 68% of non-biomass recovered ^{14}C in the legume- and wheat-amended soils respectively were present in the Lf $>250\ \mu m$). About 20% of the recovered non-biomass ^{14}C was associated with the silt-size fraction (Hf 2–50 μm) and about 10% with the clay-size fraction.

During the period 3–66 d, net decreases of non-biomass ^{14}C were calculated for soils amended with plant residues. Non-biomass ^{14}C accounted for 29 and 36% of input ^{14}C in soil amended with legume and wheat, respectively, after 66 d incubation. By the end of the incubation, non-biomass ^{14}C of the legume- and wheat-amended whole soil had declined by 23 and 30%, respectively; in the light fraction, Lf $>250\ \mu m$, by 80 and 63% respectively; and in the light fraction, Lf 50–250 μm, by 44 and 20% respectively. Non-biomass ^{14}C of the silt-size fraction (Hf 2–50 μm) increased during incubation.

DISCUSSION

Substrate decomposition

Values for evolved CO_2 ^{14}C after 3 d were equivalent to 42, 37, 27 and 16% of input ^{14}C in soils amended respectively with glucose, starch, legume and wheat. On the assumption that soluble ^{14}C of plant residues (equivalent to 22% of plant residues ^{14}C) was mineralized to an extent no greater than glucose ^{14}C (42% of input glucose ^{14}C), then CO_2 ^{14}C from decomposition of soluble plant ^{14}C was equivalent to no more than 9% of plant residue ^{14}C. These results indicated that some insoluble components of the plant residues were also decomposable, in agreement with Reinertsen *et al.* (1984). Such components will include proteins, which were more abundant in the legume residues than in the wheat material. Soluble ^{14}C components of the plant residues may be decomposed either whilst in association with the particulate residues, or with soil inorganic components, after diffusion from their original location with the residues.

At the end of the incubation, CO_2 ^{14}C represented respectively 63, 64, 59 and 51% of input ^{14}C for soil amended with glucose, starch, legume and wheat material. Similar extents of glucose decomposition were obtained by Ladd *et al.* (1992) using the same Vertisol, incubated at 40% WHC. Net average decay rates of residual organic ^{14}C from the period 3–66 d were highest in the soils amended with starch or legume ($9.1\ 10^{-3}$) and decreased in soils amended with wheat ($8.5\ 10^{-3}$) and glucose ($7.3\ 10^{-3}$). Glucose disappeared entirely during the first 3 d (van Veen *et al.*, 1985; Ladd *et al.*, 1992), and microbial products of its metabolism were therefore less decomposable on average than were plant plus microbial residues from the early decomposition of legume and wheat materials.

The efficiency of utilization of substrate ^{14}C by decomposer organisms, when expressed by the ratio biomass ^{14}C-to-the sum of biomass ^{14}C and CO_2 ^{14}C, were similar after 3 d, and ranged from about 0.57 in the glucose- and legume-amended soils to about 0.64 in the starch- and wheat-amended soils. These growth efficiencies were consistent with those listed by McGill *et al.* (1981). The slight differences in the calculated growth efficiencies may have resulted at least in part from differences in the properties of the substrate metabolized and in the energy demands by different decomposer populations. The value for this ratio suggested that, under the conditions used, little or no turnover of ^{14}C through the biomass had occured for any added substrate in the Northfield soil within 3 d. However, values decreased on extended incubation, indicative of turnover of ^{14}C. At 66 d, growth efficiencies differed between soils amended with soluble substrates (40%) and plant residues (24%).

After 3 d values for the ratio, biomass ^{14}C-to-CO_2 ^{14}C ranged from 1.8 in the starch-amended soil to 1.3 in the legume-amended soils. At the end of the incubation, this ratio was about 0.40 in the soils amended with soluble substrates, and 0.24 in the soils amended with particulate plant residues substrates. As argued by Ladd *et al.* (1995a), the higher value in the former cases, may be due to a higher average protection of biomass derived from soluble substrates than that derived from particulate residues. Comparisons of the ratio calculated for glucose and legume metabolism, with those reported for these substrates by Ladd *et al.* (1995a) demonstrated some similarities in ^{14}C turnover. However, the turnover of legume ^{14}C in our study was delayed compared with that reported by Ladd *et al.* (1995a) and the stimulating effect of particulate plant residues on total biomass C was shorter than that measured by Sørensen *et al.* (1996). Differences in soluble C content and predation pressures (Kuikman and Van Veen, 1989), due to differences in soil bulk density and moisture content respectively, may explain these results.

During the period 3–66 d, substrate-derived biomass ^{14}C decreased in each treatment. Net biomass ^{14}C decline, when expressed as a percentage of biomass ^{14}C accumulated after 3 d, ranged from 72% in the soil amended with starch to 66 and 54% in soils amended with particulate substrates, and glucose, respectively. For the glucose and starch treatments, rates of conversion of dead biomass ^{14}C-to-CO_2 ^{14}C were higher (0.90 and 0.70, respectively) than those commonly found (Gregorich *et al.*, 1991). These high rates may have arisen from the

presence of sufficient soluble (glucose and starch) substrates to sustain bacterial growth and therefore preferential utilization of recent ^{14}C-labelled assimilates for respiration (Bremer and Kuikman, 1994). In contrast, evolved CO_2 ^{14}C was higher than dead biomass ^{14}C in soils amended with particulate substrates, net decline of biomass ^{14}C may obscure synthetic and catabolic processes.

Priming effect

Brookes *et al.* (1990) pointed out that the priming effects of added substrates on soil organic matter decomposition may falsely result from experimental conditions such as high bicarbonate concentrations in soil solution, and the use of non-uniformly labelled substrate amendments or large substrate additions. None of these conditions was applicable in our experiment. True priming effects, the stimulation of the decomposition of non-biomass ^{12}C compounds, may result in part from the accelerated death of biomass ^{12}C (Dalenberg and Jager, 1989; Mary *et al.*, 1993).

In our experiment, the highest amounts of primed CO_2 ^{12}C were recorded during the first 3 d when primed ^{12}C represented 115, 35, 75 and 65 $\mu g\ C\ g^{-1}$ soil in soils amended with glucose, starch, legume, and wheat, respectively. After 3 d in soils amended with soluble substrates, concentrations of total biomass C were the same as that of the unamended control soil, even though about 60% of the total biomass C of amended soils was derived from added substrates. Thus, large amounts of indigenous biomass ^{12}C had disappeared (580 and 610 $\mu g\ C\ g^{-1}$ soil in the glucose- and starch-amended soil) during the early period of rapid metabolism. In contrast to soils amended with soluble substrate, concentrations of total biomass C increased in soils amended with plant residues, to be 1.3 times (legume-amended soil) and 1.2 times (wheat-amended soil) that in the control. Because the enhanced biomass C resided within the plant remnants, there were little opportunities for substitution of indigenous biomass ^{12}C by biomass ^{14}C within the soil matrix, as indicated by small amounts of biomass ^{12}C disappearing (100 and 150 $\mu g\ C\ g^{-1}$ soil in the legume- and wheat-amended soils, respectively) during the early stage of decomposition. These results were consistent with the theory proposed by Ladd *et al.* (1995b) where a soil has a given capacity to protect micro-organisms. Above this threshold, biomass C within the soil matrix is rapidly preyed upon. Unlike plant residue amendments, soluble substrates migrate within the soil matrix and did not offer new sites for micro-organisms, then leading to much more available indigenous biomass ^{12}C for predation and mineralization.

However, this view of a priming mechanism is weakened by the evidence that there had been no turnover of biomass ^{14}C at the time of decline of biomass ^{12}C and the priming of ^{12}C mineralization. Five explanations can be proposed:

1. Some substrate ^{14}C was rapidly incorporated into the cell constituents without net cell growth, accompanied by some decomposition of replaced ^{12}C constituents and decreases in measured biomass ^{12}C.
2. Newly formed biomass ^{14}C did not equilibrate with biomass ^{12}C such that only biomass ^{12}C became unprotected and converted to CO_2 ^{12}C.
3. Growth of predominantly biomass ^{14}C caused a disproportionately higher death of biomass ^{12}C owing to reasons other than predation (substrate, oxygen deficit, or antibiosis), resulting in some biomass ^{12}C conversion to CO_2 ^{12}C, whereas biomass ^{14}C was totally unaffected.
4. Turnover of biomass ^{14}C may have been underestimated because of an overestimation of biomass ^{14}C, despite a downward adjustment of the $K_{^{14}C}$ factor to allow for the effect of soil moisture content on the biomass assay.
5. Primed ^{12}C does not originate exclusively from biomass ^{12}C decomposition: some CO_2 ^{12}C may have originated from non-biomass ^{12}C.

Sites of microbial assimilation and turnover

Physical fractionation procedures may separate soil into components comprised of nearly discrete entities. For example, Bruckert's method for soil dispersion, although less gentle than that used by Chotte *et al.* (1992, 1993), permitted the subsequent separation of particulate plant residues from sand-size particles and stable microaggregates, with a full recovery of microbial biomass C. Microscopic examination of Lf $>250\ \mu m$ and Lf 50–250 μm from the Northfield clay soil indicated that they were mainly comprised of plant fragment remnants, increasing respectively in their stage of decomposition. Unlike Lf $>250\ \mu m$, the fraction Lf 50–250 μm, contained a few small aggregates and fine sand-size particles. Although the nature of these aggregates was not studied, they may have been small plant fragments occluded in clay aggregates (Golchin *et al.*, 1994). The silt-size fraction (Hf 2–50 μm) accounted for 50.8% of total soil weight. Jocteur Monrozier *et al.* (1991) showed that 75% of this fraction was comprised of microaggregates. A minority (35%) of soil clay was dispersed by the Bruckert method. The fraction Hf $>50\ \mu m$, consisted entirely of sand-size particles. This fraction, as obtained by the Bruckert method, accounted for the same proportion of soil as sand particles ($>50\ \mu m$ in dia.) obtained by conventional mechanical analysis after the removal of organic matter and the complete dispersion of the soil.

Incubation time, 3 d Most of the recovered biomass ^{14}C of soils amended with the soluble substrates, glucose and starch, resided in the silt-size fraction and represented, respectively, 66 and 63% of the total biomass C of this fraction. Presumably, metabolism of these substrates started after their diffusion within the soil matrix where organisms were located at sites principally on and within clay microaggregates. In contrast, substrate-derived biomass ^{14}C of soils amended with plant residues was bimodally distributed, with peak amounts associated with the fractions, Hf 2–50 μm and Lf >250 μm. The amounts of biomass ^{14}C associated with Lf >250 μm approximated the extra amount of biomass C present in the plant residue-amended soils compared with the unamended control. The oven-dried plant residues provided attractive sites for colonization by soil micro-organisms and, based on the amounts of CO_2 ^{14}C, microbial activity was directed towards both soluble and insoluble compounds in the early phase of decomposition. The exact sites of decomposition cannot be proven but they appeared to be within the plant residues and within the near vicinity of these residues. Biomass ^{14}C located in the silt-size fraction (Hf 2–50 μm) was probably formed from the metabolism of soluble ^{14}C compounds which diffused to the surfaces of clays present in microaggregates. Biomass ^{14}C of the clay-size fraction (Hf 0–2 μm) may have derived from the displacement of clay-size particles from larger sand-size components and microaggregates during the dispersion (Chotte *et al.*, 1992).

Incubation period, 3–66 d Irrespective of soil amendment, biomass ^{14}C, as a percentage of residual organic ^{14}C, declined in each fraction. The decline was due more to a decrease of biomass ^{14}C than to an increase of non-biomass ^{14}C. Also, irrespective of substrates, biomass ^{14}C, within plant remnants (Lf >250 μm) after 3 d, of incubation essentially disappeared after 66 d. Net declines in biomass ^{14}C, expressed as percentages of biomass ^{14}C at 3 d, ranged from 30 to 70% for other fractions and were not related to the nature of substrate amendments.

Most (>50%) of the decline in biomass ^{14}C of unfractionated soils amended with soluble substrates was due to the decline in biomass ^{14}C of the silt-size fraction (Hf 2–50 μm), whereas the decline in biomass ^{14}C in soils amended with particulate plant residues was due mainly to that located in plant residues (Lf >250 μm).

Net changes of non-biomass ^{14}C of fractions were recorded mainly in Lf >250 μm and Hf 2–50 μm for soils amended with particulate substrates. Non-biomass ^{14}C decreased in Lf >250 μm due to the continued decomposition of plant remnants. Non-biomass ^{14}C increased in the silt-size microaggregate fraction, Hf 2–50 μm (and to a lesser extent in the clay-size fraction, Hf 0–2 μm), due to the continued turnover of biomass ^{14}C from all fractions, and the stabilization of microbial ^{14}C-labelled products and debris by their interaction with clay. The same trend for accumulation of non-biomass ^{14}C in Hf 2–50 μm was also observed for soils amended with glucose and starch.

CONCLUSION

The decomposition of soluble or particulate substrates incorporated into a highly aggregated Vertisol resulted in the growth and turnover of biomass at different sites within the soil matrix. Site differences were inferred from the differences in the growth and the protection of biomass ^{14}C located with size fractions of the soil during the two main important phases of the incubation, viz. the 0–3 d period when there was no ^{14}C turnover and the 3–66 d period when ^{14}C turnover was evident.

By definition, a site is favourable for microbial growth if it shelters more than 50% of soil biomass at 3 d incubation. Thus, microaggregates 2–50 μm are favourable sites in soils amended with soluble substrates, whereas organic residues >250 μm represent the most favourable sites in soils amended with particulate plant residues. Moreover, newly formed biomass sheltered in microaggregates tended to replace indigenous soil micro-organisms. This substitution lead to extra CO_2 C because microbial biomass is above the protective capacity of the soil leading to an enhanced predation and conversion of some biomass ^{12}C to CO_2 ^{12}C (Ladd *et al.*, 1996). Substitution is less likely to occur in particulate amended soils, where soil micro-organisms colonized amended residues. Moreover, this newly formed biomass C is responsible for the increase of total biomass C.

A site is referred to as protective if its biomass decline, during the 3–66 d period, represents less than 50% of its biomass at 3 d of incubation. Organic residues >250 μm are not a protective site, their biomass disappearing almost entirely irrespective of the nature of the substrate.

If we combine these two properties, we observe that the main difference between treatments emerged in the fractions, organic residues (>250 um) and the microaggregates (2–50 μm). Organic residues are non-favourable and non-protective sites, microaggregates are favourable in soluble substrate-amended soils only, but non-protective.

Particulate organic residues and microaggregates 2–50 μm are the two main sites of soil organic matter decomposition and the responses of the associated biomass at these sites varied according to the nature of the substrate. Soluble substrates (e.g. rhizodeposits) promote decomposition within microaggregates, without stimulating total soil biomass. This accommodates the view that a soil has a given

capacity to shelter micro-organisms (Ladd *et al.*, 1996). The incorporation of fresh particulate organic residues (e.g. roots, leaves) temporarily increases soil biomass by offering new sites for micro-organisms. The importance of organic residues in processes of soil organic matter decomposition are then reinforced, these residues contributing to a large extend to the pool of inorganic N (Feller, 1993). As argued by Ladd *et al.* (1995a), one should therefore address the opportunity to split the biomass compartment of most models into two different pools, one associated with fresh particulate organic matter the other stabilized within existing microaggregates, these compartments having different residence time (Buyanovsky *et al.*, 1994).

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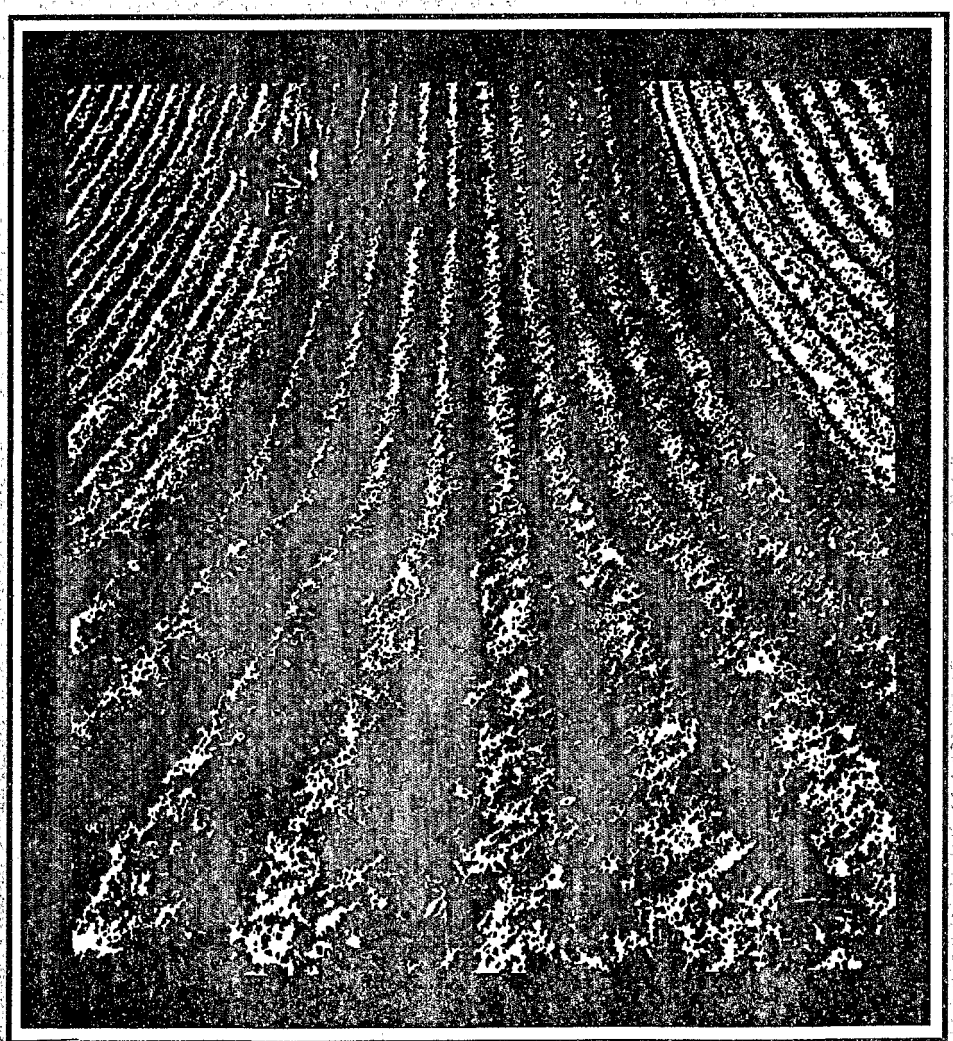

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