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Short-term utilisation of ^{14}C-[U]glucose by soil microorganisms in relation to carbon availability

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Abstract

The release of organic compounds into the soil by plant roots increases the availability of carbon in the rhizosphere and consequently stimulates the growth and the activities of microorganisms. Therefore, the determination of carbon availability in soils is a key point to better understand nutrient flows. We investigated the short-term utilisation of ^{14}C-[U]glucose by soil microorganisms to determine whether it could evidence differences in carbon availability between unplanted and maize-planted soils. In unplanted soils, the kinetics of glucose uptake indicated a multicomponent carrier-mediated transport. The lower affinity constant (K_m) for glucose uptake was determined at 74 μM of glucose. The kinetics of $^{14}CO_2$ production from unplanted soil amended with 0.07 μg C-glucose g^{-1} soil demonstrated a peak of respiratory activity during the first 15 min. One hour after ^{14}C-glucose addition, the mineralisation of the substrate represented 7.8% of the ^{14}C that was unrecovered by 0.5 M K_2SO_4 extraction and which was assumed to be absorbed by soil microorganisms (97% of the ^{14}C amended). Three days after ^{14}C-glucose addition, $^{14}CO_2$ increased to 28% of the absorbed glucose in unplanted soils. The ^{14}C extractable by K_2SO_4 after fumigation with chloroform remained constant with time (about 25%). The apparent high efficiency of utilisation of ^{14}C suggested that under conditions of carbon limitation, the substrate is rather stored by microorganisms than used for growth. We proposed a model derived from the one of Bremer and Kuikman (Soil Biol. Biochem 26 (1994) 511) for the short-term uptake and utilisation of glucose by soil microorganisms. Furthermore, we found that the utilisation of ^{14}C-glucose by microorganisms was significantly different between unplanted and maize-planted soils. In planted soils, microorganisms mineralised a significant larger proportion of the absorbed glucose (32%) and the ^{14}C-activity in the fraction released after fumigation by chloroform vapours was lower (22%). This indicated a greater availability of carbon in maize-planted soils, which was related to the release of organic compounds by roots.

Keywords: ^{14}C[U]-glucose; Available carbon; Kinetics; Rhizosphere; Maize

1. Introduction

Plant roots are a source of organic compounds, which sustain microorganisms in the rhizosphere (Curl and Truelove, 1986). Since microbial activities regulate nutrient cycling in soils (Scow, 1997), a major attention is paid to the pool of carbon available to microorganisms (AC). Sikora and McCoy (1990) and Bradley and Fyles (1995a) have suggested that AC can be estimated by extrapolating negatively the hyperbolic Michaelis–Menten relationship between glucose added to the soil and the microbial respiratory activity, to the point where the respiratory activity is zero. It is proposed that the negative substrate concentration where respiration is zero is the index of AC. Bradley and Fyles (1995b) suggested that this method indicated a greater pool of AC in planted soils compared to unplanted ones. However, the key point of the AC determination by this method is the assumption that a one component Michaelis–Menten relationship holds across all the rates of glucose addition (Badalucco and Hopkins, 1997). This could be put into question because there is ample evidence in the literature that the affinity of the glucose uptake system may change with the sugar concentration (Gottschal, 1992). In the yeast *Saccharomyces cerevisiae*, high- and low-affinity systems have been evoked but from recent work, it became clear that glucose modulates the affinity of the transporter (Walsh et al., 1994; Boles and Hollenberg, 1997). In addition, the estimates of AC obtained with different added substrates (glucose, glutamine) were not correlated (Badalucco and Hopkins, 1997).

By using ^{14}C-glucose, Bremer and van Kessel (1990) investigated the relationship between glucose addition (30 and 300 μg C g^{-1} soil) and utilisation of the sugar by soil microorganisms. At 30 μg C g^{-1} soil, they showed that 1, 3 and 7 days after the substrate addition, less C from glucose

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was respired and more of it was assimilated and recovered in a chloroform-labile pool compared to 300 μg C g^{-1} soil. Bremer and Kuikman (1994) suggested the following conceptual model. Once acquired by soil microorganisms, the substrate enters a small intermediate pool from where it may be respired or incorporated into either a conserved chloroform-labile pool or structural compounds (not thought to be liberated by chloroform) if there is sufficient substrate to sustain growth. Therefore, these authors suggested that such response could be used as an indicator of AC in the rhizosphere. In the vicinity of growing roots, AC is greater than in the bulk soil because roots release photosynthetically derived carbon in a variety of soluble (exudates) and insoluble (sloughed-off cells and root debris) forms, the totality of which is referred to as rhizodeposition. Therefore, we hypothesise that the utilisation of glucose by microorganisms should be different in planted soils compared to unplanted soils. In the former soils, the added substrate should be more mineralised and less recovered in the chloroform labile fraction compared to the latter soils. The present work aimed to examine this hypothesis. First, we tested the relevance of the conceptual model proposed by Bremer and Kuikman (1994) by investigating the short-term uptake and utilisation of ^{14}C-glucose by soil microorganisms. Secondly, we examined the differences of ^{14}C-glucose utilisation by microorganisms in unplanted and maize-planted soils.

2. Materials and methods

2.1. Soil and maize cultures

The soil was collected (0–30 cm depth) at a field site near Nancy, North-East of France. It had a clay loam texture (26.1% clay; 37.6% loam; 34.2% sand). It was air dried, sieved (5 mm) and mixed with 2 mm-sieved river sand (2:1 v/v, respectively). The pH(H_2O) was 6.5. The total organic C was 12.9 mg C g^{-1} DW soil and the soluble C was 204.3 μg C g^{-1} DW soil. The microbial C was determined at 131.6 μg C g^{-1} DW soil based on the fumigation-extraction method (Vance et al., 1987) using a k_{EC} of 0.45 (Wu et al., 1990). Soil (2 kg DW) was packed into PVC pipes (height 40 cm; internal diameter 7.5 cm) the bases of which were covered with a nylon tissue (50 μm-mesh size). In the case of planted soils, a single maize seed (cv DEA, Pioneer, France) was sown in each pipe. The soil surface was covered with a 2-cm deep bed of gravel (size: 5–10 mm). Both unplanted and planted pipes were maintained at 75% of the soil water holding capacity. The planted soils were supplied with Hoagland nutrient solution twice a week at the following rates: 20 ml $plant^{-1}$ for the first two weeks then 40 ml $plant^{-1}$ until harvest. Twenty ml of nutrient solution was added to the unplanted soils after two weeks. Planted and unplanted pipes were kept in a greenhouse under the following conditions: 25 ± 2°C/20 ± 2°C day/night temperatures, 16 h photoperiod at 300 μmol m^{-2} s^{-1} in the photosynthecally active range. Twice a week, pipes were moved round to ensure homogenised culture conditions. Soil from both the planted and unplanted pipes was collected after 6 weeks of culture. At this time, the maize plants had 11 visible leaves and 7 unfolded ones. The visible roots were carefully separated from the soil by hand and washed with tap water. Immediately after collecting the soil, it was homogenised, packed into plastic bags (around 700 g per bag) and kept at −20°C until analysis to minimise changes in the activities and in the size of microbial biomass (Stenberg et al., 1998). For incubation with ^{14}C-glucose, subsamples were taken as soon as the soil was thawed. The water content of all soils was determined by drying at 70°C for three days.

2.2. ^{14}C-Glucose uptake assay

^{14}C-D-glucose uniformly labelled glucose (specific activity: 7400 MBq $mmol^{-1}$) was added to unplanted soils. At the onset, 2 ml of a ^{14}C-glucose solution was mixed with 2 g of soil using a magnetic stirrer at 22°C. The amounts of glucose added (μg g^{-1} FW soil) and the specific activities (values in brackets in kBq mg^{-1} C) were: 0.1 (41066); 0.3 (481); 0.6 (481); 1.2 (481); 2.4 (481); 4.8 (481); 9.6 (481); 19.2 (481); 38.6 (123); 58.0 (29); 77.1 (62); 116.0 (29); 154.2 (31). There were three replicates for each concentration of added glucose. All solutions were made from ultrapure water. The ^{14}C-activity in the soil solution was monitored as follows. For each glucose concentration, nine 0.2 ml aliquots of the slurry were sampled over 4.5 min at 30 s intervals and each aliquot was mixed with 0.5 ml of lactic acid 0.2 M in order to stop glucose uptake by microbial biomass and to liberate ^{14}C-carbonate. In a separate experiment, we determined the amount of soil contained in 0.2 ml aliquots of the slurry. It was 32.2 mg DW soil ± 1.54% ($n = 9$). The samples were centrifuged for 5 min at 10000g. The ^{14}C-activities of the supernatant as well as the activity of the initial glucose solutions were determined from a 0.5 ml aliquot by liquid scintillation counting using Ultima Gold scintillation cocktail (Packard). The radioactivity remaining in the soil solution was plotted against time. The relationship was linear for the first 120 s. The slope gave the absolute value of the rate of glucose uptake by microorganisms.

In a separate experiment, the kinetics of glucose uptake was monitored for 1 h. Soil samples (4 g FW) were mixed with 4 ml of a ^{14}C-glucose solution (30.7 kBq, 4.1 nmol glucose corresponding to 0.07 μg C-glucose g^{-1} soil). We sampled 16 aliquots of 0.2 ml and ^{14}C-activity remaining in the soil solution was determined as described above. The experiment was replicated three times.

2.3. Kinetics of $^{14}CO_2$ production in unplanted soils incubated with ^{14}C-glucose

A flow of CO_2-free air was delivered from a pressurised

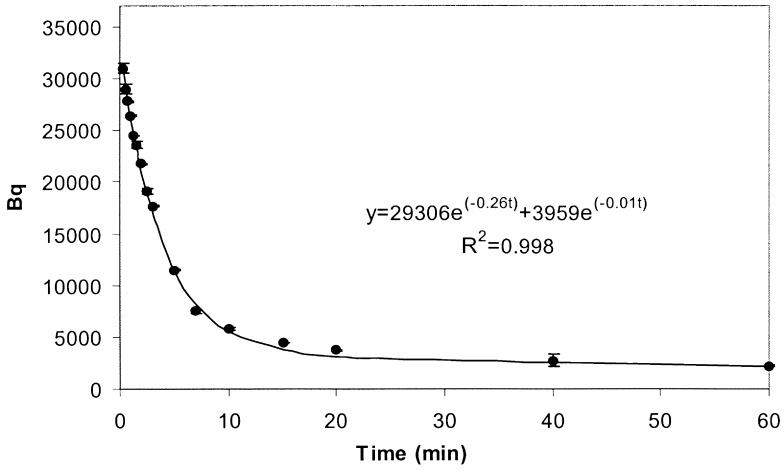


Fig. 1. Kinetics of ^{14}C-activity depletion in the solution of unplanted soil amended with 0.07 μg C-glucose g^{-1} soil. Vertical bars when visible indicate standard error ($n = 3$).

cylinder and was regulated at 45 ml min^{-1} by a mass flowmeter. The air passed through a 20 ml glass vial containing 4 g of fresh soil, through a 20 ml cartridge filled with Drierite™ as a desiccant followed by a 50-mm diameter air filter (size pore: 0.2 μm) and through the 15 ml cell of a radioactivity detector. The cell is made of scintillant plastic that allows the flow detection of beta radiation (Benoit et al., 1994; Nguyen et al., 1999). The outflow of the cell bubbled into NaOH 1 M in order to trap waste of $^{14}CO_2$. A 100 μl aliquot of ^{14}C-glucose solution (30.7 kBq, 4.1 nmol glucose, 0.07 μg C g^{-1} soil) was spread over the soil and the vial was plugged immediately. The mineralisation of ^{14}C-glucose was monitored for 1 h. Afterwards, the unabsorbed ^{14}C in the soil was determined by extraction for 45 min at 40 rev min^{-1} with 16 ml of 0.5 M K_2SO_4. After filtration on a Whatman No. 42 paper disc, the activity in the K_2SO_4 extract was determined by liquid scintillation counting of a 1 ml aliquot (Ultima Gold scintillator, Packard).

2.4. Short-term utilisation of ^{14}C-glucose by soil microorganisms

Treatments consisted in the comparison of ^{14}C-glucose utilisation by soil microorganisms between maize-planted and unplanted soils after 1 h and 3 days of incubation. All treatments were replicated four times. Soil replicates corresponded to distinct plants or soil pipes. Two 25 ml glass vials filled with 4 g FW soil (aimed to be fumigated and non-fumigated soils) were installed within a 1 l glass jam jar containing 50 ml of 1 M NaOH to trap $^{14}CO_2$. A 0.5-ml aliquot of a ^{14}C-glucose solution (4.1 kBq, 0.01 μg C-glucose g^{-1} soil) was spread over the soil surface in each vial and the jar was instantaneously closed. A rubber gasket with vacuum silicon grease was fitted to the cover to prevent leaks. The incubators were installed in the dark at 22°C for 1 h or for 3 days. The $^{14}CO_2$ was determined by liquid scintillation counting of 1 ml of the NaOH solution mixed with 10 ml Ultima Gold (Packard) scintillation cocktail.

After the incubation, the non-fumigated soils were directly extracted with K_2SO_4 and the soils aimed to be fumigated were installed within a glass dessicator with ethanol-free chloroform. The vacuum was made until the chloroform boiled for 5 min. Then, the dessicator was maintained under vacuum in the dark for 24 h at 25°C. Afterwards, chloroform vapours were removed by evacuation six times (−0.09 MPa). Fumigated and non-fumigated soils were extracted with 16 ml of 0.5 M K_2SO_4 and ^{14}C was determined as described previously.

2.5. Calculation and statistics

We determined the partitioning of ^{14}C as follows:

- $^{14}C_0$: ^{14}C-glucose added, determined by counting the ^{14}C-glucose solution (kBq);
- $^{14}C_{UNABS}$: ^{14}C in the 0.5 M K_2SO_4 extract of non-fumigated soils;
- $^{14}C_{ABS}$:^{14}C absorbed = $^{14}C_0 - {}^{14}C_{UNABS}$;
- $^{14}CO_2$: (^{14}C in the NaOH solution or as determined by the flow detection of radioactivity) × 100/ $^{14}C_{ABS}$
- $^{14}C_{FE}$: (^{14}C in the 0.5 M K_2SO_4 solution after the chloroform fumigation − $^{14}C_{UNABS}$) × 100/$^{14}C_{ABS}$;
- $^{14}C_{MB}$: Microbial $^{14}C = (^{14}C_0 - {}^{14}C_{UNABS} - {}^{14}CO_2) \times 100/{}^{14}C_{ABS}$;
- $^{14}C_{non\text{-}FE}$: ($^{14}C_{MB}$) − ($^{14}C_{FE}$);
- ^{14}C efficiency = $^{14}C_{MB}/(^{14}C_0 - {}^{14}C_{UNABS})$;
- k_{EC}: proportion of microbial ^{14}C which is released by the fumigation with chloroform = $^{14}C_{FE}/{}^{14}C_{MB}$.

Kinetics of glucose uptake and Michaelis–Menten models were fitted to experimental data by non-linear regression using the nlinfit function of MATLAB v5.2 (Mathworks, Inc.). Data were analysed by two-way ANOVA

Table 1
^{14}C-activity ± standard error ($n = 3$) in the soil solution and in respiration of soil amended with ^{14}C-glucose (0.07 μg C-glucose g^{-1} soil, 30.7 kBq) as a function of time. Parameters of the relationship fitted on experimental data $y = A_1\, e^{-\alpha t} + A_2\, e^{-\beta t} + A_3\, e^{-\gamma t}$ obtained for the first hour following ^{14}C-glucose addition. (Within a given section (constant terms, specific velocities), values with the same upperscript letter are not significantly different (Newman Keuls test, $p = 0.05$, $n = 3$))

		^{14}C in the soil solution (q_1)	Rate of ^{14}C-mineralisation ($d^{14}CO_2/dt$)
Constant terms (Bq)	A_1	29 306^{a} ± 347.5	6341^{c} ± 221.1
	A_2	3959^{b} ± 369.3	50^{d} ± 1.3
	A_3		−6429^{e} ± 226.2
Specific velocities (min^{-1})		0.26^{a} ± 0.011	0.29^{a} ± 0.013
		0.01^{b} ± 0.005	0.02^{b} ± 0.008
			0.30^{a} ± 0.014
R^2		1.00 ± 0.001	0.95 ± 0.002

using STATITCF software. The Newman–Keuls test was used for mean comparisons at 5%.

3. Results and discussion

3.1. Kinetics of ^{14}C-glucose uptake by microbial biomass of unplanted soils

Immediately after ^{14}C-glucose addition, ^{14}C-activity in the soil solution decreased with time (Fig. 1). We assumed this reflected only the microbial uptake. Indeed, in a separate experiment, triplicates of 20 g DW soil at 75% of the soil water holding capacity were autoclaved twice at 121°C for 20 min before being supplied with ^{14}C-glucose (1250 Bq each). After 1 h, 95 ± 0.6% were recovered from the soils after extraction with K_2SO_4 0.5 M. As previously reported (Darrah, 1991; Jones and Edwards, 1998), glucose was not sorbed to soil particles, as it is uncharged.

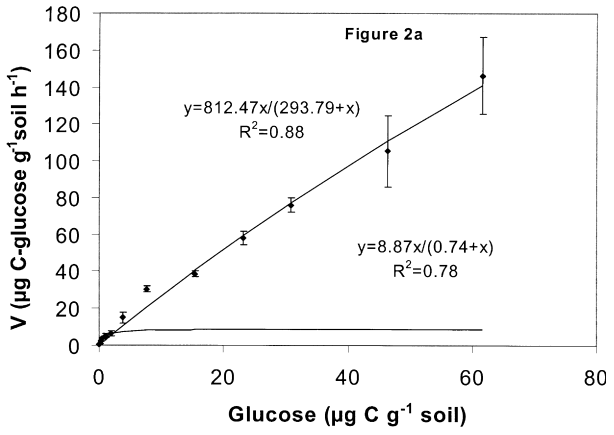
The kinetics of glucose uptake fitted a sum of two exponentials (Fig. 1) whose specific velocities differed by a factor of 26 (Table 1). The initial velocity of ^{14}C-uptake plotted versus glucose concentrations (Fig. 2a) fitted a Michaelis–Menten model $V = V_{max}S/(K_m + S)$ ($R^2 = 0.88$). The K_m value was 294 μg C-glucose g^{-1} fresh soil (735 μg glucose g^{-1} soil, 29 mM glucose in the soil solution). If the relationship had been described by a one-component Michaelis–Menten model, $V = -K_m(V/S) + V_{max}$, the Eadie–Hofstee plot of ^{14}C-glucose uptake kinetics (V against V/S) would have been linear, but this was not the case (Fig. 2b) which suggests the involvement of at least two systems for the uptake of glucose. We examined the data obtained for glucose ranging between 0 and 2 μg C-glucose g^{-1} soil (Fig. 2c). For those concentrations of glucose, a one-component model gave a K_m of 0.7 μg C-glucose g^{-1} soil (1.8 μg glucose g^{-1} soil) which corresponded to a K_m of 74 μM glucose. Extrapolation of the one-component model of the glucose uptake indicated that V_{max} was reached at 6 μg C-glucose g^{-1} FW soil. By comparison, in *Saccharomyces cerevisiae*, the K_m of the high affinity system of glucose uptake is about 0.7 mM under derepressing conditions (Boles and Hollenberg, 1997). Recent works suggested that there is a complex genetic control of the affinity of glucose transporters rather than two separate low- and high-affinity systems (Reifenberger et al., 1997).

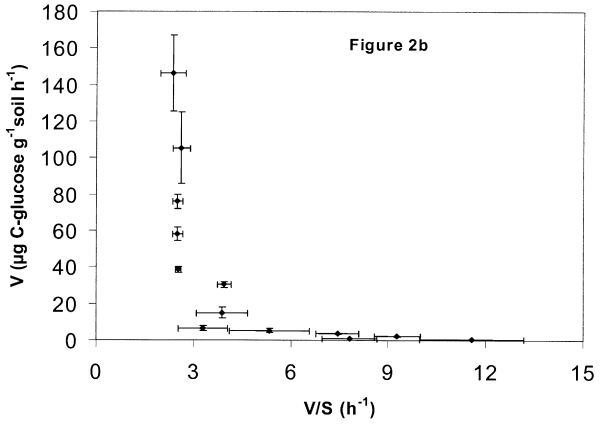
In case of soil microbial populations, the K_m for the glucose uptake was determined at 2.4 mM by Coody et al. (1986). The lowest concentration of glucose used by these authors was 2.5 mM and was not adapted to point out a high-affinity system. Indeed, in our work, the high affinity system (K_m of 74 μM) was observed for glucose below 200 μM.

Plant roots are able to uptake organic compounds such as sugars, amino acids and organic acids (Schobert and Komor, 1987; Jones and Darrah, 1992). This process can be interpreted as a regulation of the passive loss of soluble carbon by exudation (Jones and Darrah, 1993, 1996). In maize root tips, two systems for the uptake of glucose were demonstrated with K_m values of 0.8 and 45 mM (Xia and Saglio, 1988). Here, the uptake of glucose by soil microorganisms demonstrated a K_m of 74 μM for glucose below 20 μM and a K_m of 29 mM for glucose concentrations up to 6 mM. Hence, microorganisms might take up glucose more efficiently than roots. Therefore, the significance of re-sorption of organic compounds in non-axenic soil needs further consideration to elucidate how the plant can regulate exudation and what are the consequences on C flux in the rhizosphere.

3.2. Short-term kinetics of ^{14}C-glucose mineralisation by microorganisms in unplanted soils

We examined the kinetics of ^{14}C-glucose mineralisation in relation with the kinetics of ^{14}C-glucose depletion in the soil solution because we think that experimental designs were comparable. The basic difference between the two experiments might have been oxygen availability because glucose uptake was monitored in soil slurry. However, since we maintained a vigorous agitation of the soil slurry, we assumed that oxygen was not limiting. We detected $^{14}CO_2$ immediately after the ^{14}C-glucose solution (30.7 kBq; 4.1 nmol glucose) was spread over the soil (Fig. 3). The kinetics indicated a peak of $^{14}CO_2$ for the first 15 min with the highest $^{14}CO_2$ evolution around 5 min, then the rate of $^{14}CO_2$ production decreased slightly with time. This priming mineralisation was concomitant with the rapid decrease of radioactivity in the soil solution (Figs. 1 and 3). The rate of $^{14}CO_2$ production during the first hour fitted a sum of three exponentials. There were no significant difference for the greatest specific velocities as well as for the lowest one between $d^{14}CO_2/dt$ function and the function that described





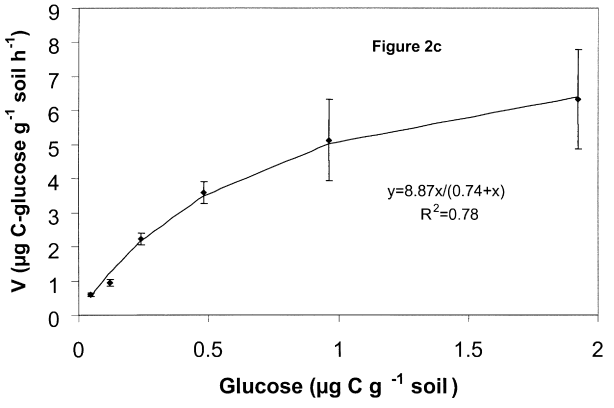


Fig. 2. Plots showing the initial velocity of glucose uptake by microorganisms of unplanted soils in relation to the rates of glucose addition: (a) illustrates the whole range of glucose concentrations in the soils and (c) concentrates on the range 0–2 µg C g^{-1} soil. Data were fitted to a one component Michaelis–Menten model; (b) is the Eadie–Hofstee plot (*V* against *V*/*S*) for the whole glucose concentrations. Vertical bars when visible indicate standard error ($n = 3$).

the radioactivity in the soil solution (Table 1). This indicated that the short-term respiration of ^{14}C-glucose might derive directly from the carbon that has just been taken up, provided that glucose adsorption on soil colloids was negligible as we assumed. Here, we demonstrated that (1) recently acquired substrate was preferentially used for respiration (2) glucose uptake depended of a multi-component system. Consequently, the relationship between the respiratory activity of soil and rates of glucose addition is not a single component Michaelis–Menten relationship as assumed by Sikora and McCoy (1990) and Bradley and Fyles (1995a) in their method to estimate AC. If a single component Michaelis–Menten relationship is assumed to hold across all the glucose concentrations, the respiratory activity of soils at low rates of glucose addition will be underestimated. Since the amount of available carbon is estimated from the extrapolation of the Michaelis–Menten model at low rates of respiration, its determination might be biased.

After 1 h, the unabsorbed glucose was 3.1% of the total ^{14}C added and the $^{14}CO_2$ represented 7.8% of the radioactivity absorbed (Fig. 3). Consequently, the efficiency of ^{14}C-glucose assimilation yielded 92%. It is hardly to be expected that within 1 h, the glucose will have been transformed into structural components of microorganisms so rapidly. It seems more likely that a fraction of the glucose was stored. This is consistent with the fact that, under starvation, bacteria can accumulate reserve polymers such as glycogen and poly-β-hydroxybutyric acid (Matin, 1992). Identical conclusions were drawn by Bremer and van Kessel (1990) and by Bremer and Kuikman (1994). In their studies, the proportion of glucose mineralised increased with the rate of glucose addition while the proportion of glucose that was recovered in the chloroform-labile fraction decreased. They suggested that at low rates of glucose addition, the sugar was conserved in the chloroform-labile pool.

In our work, the rate of $^{14}CO_2$ production during the first hour by unplanted soils determined either from a closed incubator (Table 2) or from a vial flushed with CO_2-free air (Table 1) were not different. Therefore, we used the relationship of Table 1 to extrapolate $^{14}CO_2$ production at day 3. This gives 9.8 ± 0.84% of the ^{14}C absorbed. However, it was determined at 28% (Table 2). Hence, it is suggested that respiration of ^{14}C derived also from a carbon pool that is separate from the one which was used during the first hour. Between 1 h and 3 days of incubation with ^{14}C-glucose, the $^{14}C_{FE}$ increased from 24% of ^{14}C absorbed to 26% in unplanted soils (Table 2). This indicated that the respiration of the labelled substrate derived from a non fumigation-extractable pool whose ^{14}C-activity dropped from 68% at 1 h to 46% at day 3.

3.3. Proposed conceptual model of ^{14}C-glucose utilisation by soil microorganisms

The model of Fig. 4 summarises the short-term fate of absorbed glucose. The substrate is taken up from the soil solution whose depletion with time indicated a sum of two exponentials. The ^{14}C enters then an intermediate pool with a rapid turnover rate from which it is partitioned between respiration, structural C, a non fumigation-extractable intermediate pool and a storage chloroform-labile C pool. The relevance of the intermediate pool (pool #2) was supported

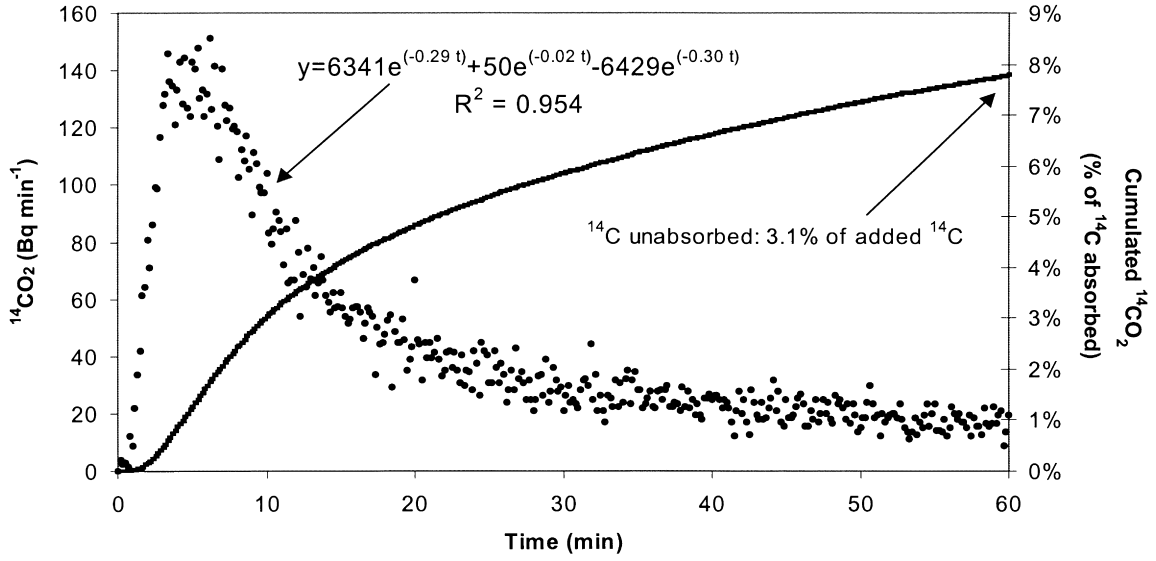


Fig. 3. Kinetics of ^{14}C-glucose mineralisation by the microbial biomass of unplanted soil (30.7 kBq, 4.1 nmol glucose, 0.07 μg C g^{-1} soil, 4 g soil). Black circles indicate the rate of mineralisation (d$^{14}CO_2$/dt) and the curve illustrated the cumulated $^{14}CO_2$ expressed as a percentage of the ^{14}C-glucose absorbed. The plot corresponds to one of the three replicates.

by the primary production of $^{14}CO_2$ for the first 15 min following the addition of ^{14}C-glucose. The non-fumigation-extractable intermediate pool (pool #4) serves for respiration and structural C. It was suggested by the decrease of the $^{14}C_{non\text{-}FE}$ between 1 h and day 3.

For the mathematical formulation of the model, we assumed that Michaelis–Menten kinetics could be simplified into first-order kinetics. This is reasonable because the amount of carbon added to the soil was very small. The transfer of ^{14}C between the pools was then formulated as follows.

In the soil solution, the radioactivity against time was fitted by:

$$q_1 = A\,e^{-\alpha t} + B\,e^{-\beta t}$$

In the chloroform-labile intermediate pool (#2), the change in ^{14}C is given by:

$$\frac{dq_2}{dt} = k_1 q_1 - (k_2 + k_3 + k_4 + k_5) q_2$$

The solution for this first-order differential equation is:

$$q_2 = C\,e^{-\alpha t} + D\,e^{-\beta t} + E\,e^{-(k_2+k_3+k_4+k_5)t}$$

In the pool #4, the change in ^{14}C-activity is:

$$\frac{dq_4}{dt} = k_2 q_2 - (k_6 + k_7) q_4$$

$$q_4 = F\,e^{-\alpha t} + G\,e^{-\beta t} + H\,e^{-t(k_2+k_3+k_4+k_5)t} + I\,e^{-(k_6+k_7)t}$$

Table 2
Utilisation of ^{14}C-glucose (0.01 μg C-glucose g^{-1} soil, 4.1 kBq) by soil microorganisms 1 h and 3 days after the glucose addition. Soils were unplanted or planted with maize for 6 weeks ($n = 4$). (Within a given line, values with the same upperscript letter are not significantly different (Newman Keuls test, $p = 0.05$, $n = 4$)

	Unplanted soils		Maize-planted soils	
	1 h	3 days	1 h	3 days
Unabsorbed ^{14}C (% ^{14}C added ± SE)	3.3^{a} ± 0.21	0.3^{b} ± 0.02	3.6^{a} ± 0.32	0.3^{b} ± 0.03
^{14}C partitioning (% ^{14}C absorbed ± SE)				
$^{14}CO_2$	7.8^{a} ± 0.71	28.0^{b} ± 0.54	8.7^{a} ± 0.20	32.2^{c} ± 1.74
$^{14}C_{FE}$	23.8^{a} ± 0.7	26.4^{b} ± 0.92	21.4^{a} ± 1.17	22.5^{a} ± 0.73
$^{14}C_{non\text{-}FE}$	68.4^{a} ± 0.18	45.6^{b} ± 0.82	70^{a} ± 1.15	45.3^{b} ± 2.26
$^{14}C_{MB}$	92.2^{a} ± 0.71	72.0^{b} ± 0.54	91.3^{a} ± 0.2	67.8^{c} ± 1.74
k_{EC} ($^{14}C_{FE}$/$^{14}C_{MB}$)	0.26^{a} ± 0.006	0.37^{b} ± 0.012	0.23^{a} ± 0.013	0.33^{c} ± 0.017

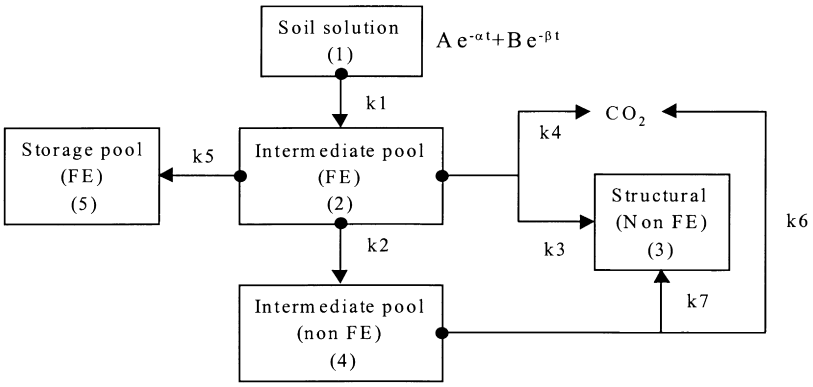


Fig. 4. Model proposed for the short-term utilisation of glucose by soil microorganisms. See text for detailed explanations.

In the chloroform labile storage pool (#5):

$$\frac{dq_5}{dt} = k_5 q_2$$

$$q_5 = J\,e^{-\alpha t} + K\,e^{-\beta t} + L\,e^{-(k_2+k_3+k_4+k_5)t}$$

The rate of $^{14}CO_2$ production is:

$$\frac{d^{14}CO_2}{dt} = k_4 q_2 + k_6 q_4$$

We hypothesised that during the first hour after the ^{14}C-glucose addition $k_6 q_4$ is negligible compared to $k_4 q_2$

$$\frac{d^{14}CO_2}{dt} = k_4(C\,e^{-\alpha t} + D\,e^{-\beta t} + E\,e^{-(k_2+k_3+k_4+k_5)t})$$

As confirmed by experimental data, the rate of $^{14}CO_2$ production is a sum of three exponentials for which two specific velocities were equal to those of the function describing the radioactivity in the soil solution (q_1) (Table 1). Between 1 h and 3 days, the intermediate pool #2 is depleted of ^{14}C and respiration of labelled substrate derived from the non-FE intermediate carbon pool (#4). The partitioning of assimilates between storage, structural and respiration reflects changes in physiology of microorganisms in relation to the availability of carbon. Under starvation conditions, the size of the cell is reduced (ultramicrobacteria) (Gottschal, 1992), the growth stops, substrates are stored rather than used for growth (Matin, 1992) and the maintenance requirements are substantially decreased (Scow, 1997).

3.4. Comparison of ^{14}C-partitioning within soil and microorganisms between unplanted and maize-planted soils

The effect of maize culture on the partitioning of ^{14}C between the soil and the microbial compartments was examined 1 h and 3 days after the addition of ^{14}C-glucose to the soils. The budget of ^{14}C after 1 h of chase-period did not indicate any significant difference between the unplanted and the maize-planted soils (Table 2). The unabsorbed radioactivity was around 3.5% of the amount brought. The respired fraction was 8.3% of the activity absorbed and the chloroform-labile pool ($^{14}C_{FE}$) was determined at 22.6% which corresponded to a k_{EC} of 24.6%. When the chase period was 3 days, the unabsorbed radioactivity, which could be extracted by K_2SO_4 was negligible in both kind of soils (0.3% of the radioactivity added). There were significant differences of ^{14}C-partitioning within the microbial biomass compartments. Expressed as a percentage of the absorbed radioactivity, the maize-planted soils demonstrated a greater $^{14}CO_2$ production, a lower allocation of carbon to the chloroform labile pool and consequently a lower k_{EC} than the unplanted soils. According to Bremer and van Kessel (1990) and Bremer and Kuikman (1994), this indicated more AC in maize-planted soils, which is consistent with the rhizodeposition of carbon. Compared to unplanted soils, the release of carbon by plant roots changed the utilisation of ^{14}C-glucose by soil microorganisms. The greater mineralisation of the substrate was explained by an increase of maintenance requirements (Scow, 1997) and an investment of the substrate in growth.

4. Conclusions

As it was hypothesised by Bremer and Kuikman (1994), our work confirmed that the pattern of ^{14}C-glucose utilisation by the soil microorganisms could be used as an indicator of carbon availability in the rhizosphere. In contrast to these authors, we did not use glucose to modify AC. By using ^{14}C-glucose with a high specific activity, we added only 0.01 μg C-glucose g^{-1} soil, which is negligible compared to the soluble carbon of the soil at the beginning of the experiment (204.3 μg C g^{-1} DW soil).

In the present work, the difference in ^{14}C-glucose mineralised between planted and unplanted soils is small (around 4%, Table 2). This may be related to the fact that in planted soils, the rhizosphere soil was mixed with the bulk soil and the ^{14}C-glucose was amended to a subsample of that mixture. Therefore, investigations could now concentrate on the improvement of the sensitivity of the method. This

can be done by injecting the ^{14}C-glucose in the vicinity of the roots (Swinnen, 1994).

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