

PRIMING EFFECT OF SMALL GLUCOSE ADDITIONS TO ^{14}C -LABELLED SOIL

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Summary—Soil was freed of its organic matter by heating it to 400°C. Plants were grown in a $^{14}\text{CO}_2$ atmosphere and from them a labelled "soil organic matter" (humus) was prepared by composting the plant material for more than 3 yr in the modified soil under laboratory conditions. The influence of small additions of unlabelled glucose on the decomposition of the labelled soil organic matter was studied. Shortly after the addition of glucose there was a small extra evolution of $^{14}\text{CO}_2$, which lasted about 1 day. It is claimed that the extra evolution of $^{14}\text{CO}_2$ was caused by conversion of labelled material in the living biomass and was not due to a real priming action, i.e. an accelerated decomposition of humic substances or dead cellular material.

INTRODUCTION

There is contradictory evidence regarding the priming effect of easily-decomposable organic amendments (as green manure) on the decomposition of native soil organic matter (Jenkinson, 1971). The evidence for a primed decomposition is based on $^{14}\text{CO}_2$ measurements after adding ^{14}C -labelled material to soil. The methodical drawbacks of this approach were summed up briefly by Jenkinson (1966a) and can be avoided by following the idea of G. W. Harmsen put forward in 1958 (personal communication) to use soil with ^{14}C -labelled soil organic matter and unlabelled additives.

We grew ^{14}C -labelled plants and by composting them in a non-organic sand-clay mixture prepared ^{14}C -labelled soil organic matter. We used glucose as an unlabelled additive.

MATERIAL AND METHODS

The plant material for the labelled humus consisted primarily of willow plants, a few maize plants and some reed, followed later by rye plants. They were grown from seedlings to maturity in a moisture- and temperature-controlled closed cabinet in a cage in the garden. The cabinet was provided with a buffer system for air expansion, a system to measure $^{14}\text{CO}_2$ in its atmosphere, a system to add CO_2 or $^{14}\text{CO}_2$ and a system to remove excess O_2 .

Fans inside the cabinet mixed the air after addition of CO_2 or $^{14}\text{CO}_2$. Periodically $^{14}\text{CO}_2$ was supplied early on bright days to get quick immobilization by photosynthesis. The plant roots were grown in vermiculite containing a nutrient solution, which was periodically replenished. All ^{14}C -labelled plant material grown in the cabinet was dried and ground and composted with a sand-clay mixture moistened to 20% by adding a soil infusion. The sand-clay mixture was prepared by heating a natural sandy loam for 2 days to 350–400°C to destroy organic matter present in the soil. The composting mixture was kept for more than 3 yr in a glass system, placed in a room in which the temperature was uncontrolled. An air pump continuously circulated air through the system, so that air moved over the surface or through the composting mixture. $^{14}\text{CO}_2$ formed was absorbed in two special washing bottles containing 25% KOH solution plus Titan yellow as an indicator. Consumed O_2 was replaced automatically by electrolytically-generated O_2 . After composting for 3 yr in the closed system the containers with the labelled soil were removed and stored at -1°C . Later the soil was thoroughly mixed and split into equal portions which were stored at about -11°C . The soil prepared in this way strongly resembles a natural sandy loam (Table 1). Though the plant material used was heterogeneously labelled, the specific activities of soil C and some fractions of it are similar which suggests that the soil organic matter was homogeneously labelled.

Table 1. Some characteristics of the "sandy loam soil" with ^{14}C -labelled organic matter

Carbon	3.25%	Specific activity: $\text{dis min}^{-1} \text{mg}^{-1} \text{C}$	
$\text{NH}_4\text{-N}$	$146 \mu\text{g g}^{-1}$	soil-C	2.7×10^4
$\text{NO}_3\text{-N}$	$150 \mu\text{g g}^{-1}$	humic acids	2.8×10^4
Moisture	20.5%	fulvic acids	2.7×10^4
pH:KCl	7.35	carbonate	2.8×10^4
CO_3^{2-}	$100 \mu\text{g g}^{-1}$		

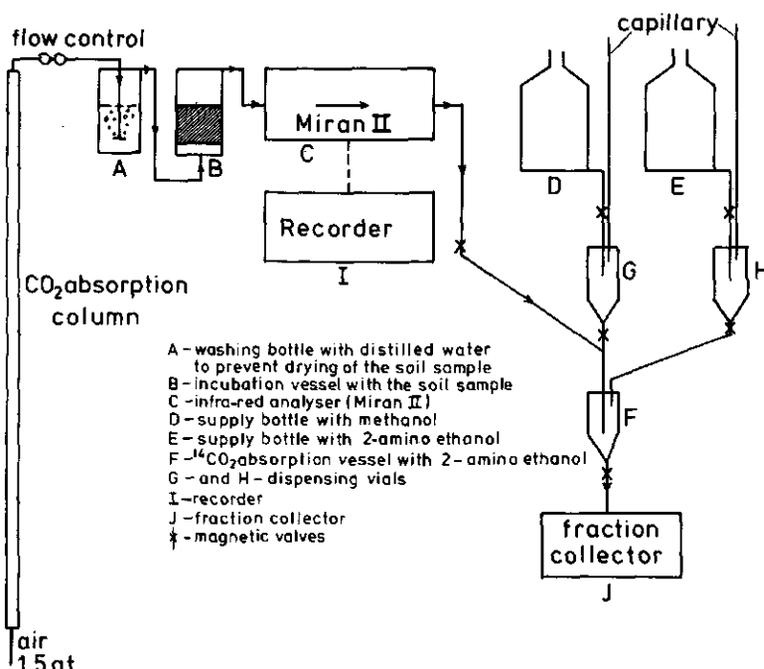


Fig. 1. Diagram of the experimental setup.

Nevertheless we have to admit the artificial character of this ¹⁴C-labelled soil.

Setup of the experiments

The experiments were performed in a closed system (Fig. 1) in a room kept at 29°C. The CO₂ evolved was measured by an i.r. spectrometer (Miran II, Wilks, U.S.A.), then absorbed in 2-aminoethanol and the ¹⁴C activity determined by liquid scintillation counting.

The tubing in the aeration system was glass. Tygon tubing was used for the addition of 2-aminoethanol and methanol. CO₂-free air was used for aeration at a constant flow (30 ml min⁻¹). Aeration with CO₂-free air, decomposed H¹⁴CO₃ in the soil so that CO₂ evolved from it could not disturb experiments carried out on it subsequently.

The incubation vessel contained 35 g labelled soil (dry-wt basis) with 1 ml soil infusion. The moisture content of the soil was about 20% (w/w). Every 3 h an absorption sample was collected. In order to avoid memory effects and to solubilize and remove 2-aminoethanol carbonate the inlet system tube was washed with methanol (Jenkinson, 1966b).

The process of aeration, emptying, washing and addition of new 2-aminoethanol and methanol in vessel F was controlled by a rotating camswitch, driven by a synchronous electric motor, also serving the magnetic valves and the fraction collector.

The absorption fluid was counted according to Bosshart and Young (1972) with an efficiency of 71% and a standard deviation of 0.5%. The small priming peaks were evaluated with 95% reliability. The standard deviation of the difference between the priming peaks and the base line, found by means of multiple regression calculations, was 8-17%.

A total volume of 200-300 μl of an aqueous solution of 250 mg glucose ml⁻¹ was added to the soil

by injecting very small portions to attain an even distribution and avoiding the effect of soil mixing (Rovira and Greacen, 1957).

During the first week of incubation the soil showed a rather strong "partial sterilization effect", probably due to decomposition of biomass killed by freezing and long cold storage. This effect lasted about 7-10 days and after about 14 days the CO₂ evolution attained a steady rate and experiments were started shortly thereafter.

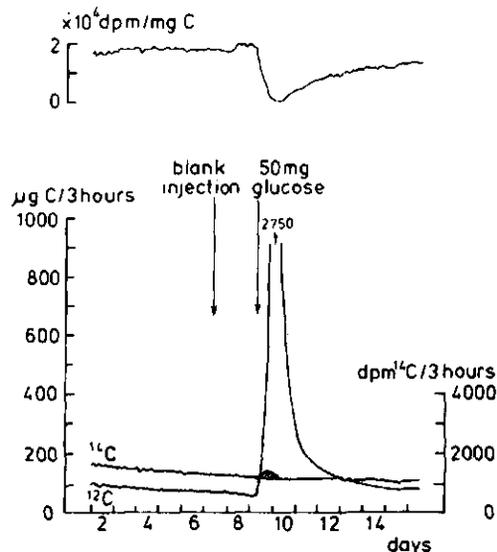


Fig. 2. The effect of addition of 50 mg glucose on the decomposition of ¹⁴C-labelled soil organic matter. ¹⁴C: labelled CO₂ from soil organic matter and biomass in dis min⁻¹ 3 h⁻¹. CO₂: total CO₂ in μg C 3 h⁻¹. The specific activity of the CO₂ evolved is given in the upper half of the figure (in 10⁴ dis min⁻¹ mg C).

Table 2. Effect of glucose additions on the decomposition of soil organic matter and the amount of glucose-C left in the soil after 7 days at 29°C

Amount of glucose added (mg)	Priming $\mu\text{g C}$	Priming % of basal metabolism*	Proportion of glucose—C
			left in the soil after 7 days
50	30	7.0 \pm 0.8%	47%
50	17	3.5 \pm 0.6%	49%
75	41	4.8 \pm 0.4%	43%

* The basal metabolism is interpolated in the ^{14}C curve (see Fig. 2) and expressed in $\text{dis min}^{-1} \text{ }^{14}\text{C } 3 \text{ h}^{-1}$.

Exchange of non-labelled CO_2 with labelled carbonate was insignificant in the soil we used. Passing a stream of air with an enhanced CO_2 content through the sample did not change the amount (content) of $^{14}\text{CO}_2$ in the outgoing air. Carbonates and hydrogen-carbonates did not give rise to errors.

RESULTS

Figure 2 shows the effect of injection of 200 μl glucose solution, containing 50 mg glucose (20 mg C). Glucose began decomposing very shortly after its addition and at about the same time $^{14}\text{CO}_2$ increased. This priming effect lasted about 1 day and was very small indeed. The total amount of ^{14}C evolved in excess of the steady state level was $30 \mu\text{g} \pm 3.3$. During the peak of glucose decomposition 10.7 mg C was lost by respiration and 9.3 mg C remained in the soil.

In the upper half of Fig. 2 the specific activity of the $^{14}\text{CO}_2$ evolved is shown. The specific activity was 1.85×10^4 disintegrations $\text{min}^{-1} \text{ mg}^{-1} \text{ C}$ and fell to nearly zero when the glucose decomposition was highest. After that it rose very gradually and again reached its original value when unlabelled substrates were exhausted. The specific activity of the respired CO_2 was lower at steady state level than the average specific activity of the soil organic matter. The same was found in the other experiment and this will be discussed below.

A second experiment in which the same amount of glucose was applied showed a smaller priming effect of $17 \mu\text{g} \pm 3$. A total of 10.1 mg glucose-C was respired during the peak. In a third experiment 75 mg glucose was added. After an immediate but small reaction there was a delay of about 6 h before the large decomposition peak began. About 6 h after the beginning of the glucose decomposition a short rise in the $^{14}\text{CO}_2$ production began and ended before the decomposition peak of the glucose had finished (Table 2).

In these experiments the effect of added glucose in producing a temporarily accelerated decomposition of labelled "native" soil organic matter was very small. Compared with the total amount of labelled soil organic matter and even with the amounts of C left in the soil from the added glucose, the extra amounts of labelled soil organic matter lost by priming were negligible.

The rapid occurrence of the priming, its coincidence with the increase in glucose decomposition and the synthesis of new biomass, and its very short duration

made us doubt whether the extra decomposition would, indeed be due to a decomposition of (stable) labelled "native" soil organic matter. We supposed the extra evolution of $^{14}\text{CO}_2$ could be due to conversions in the biomass. To distinguish between conversions in the biomass and a real priming effect, we killed the biomass by a series of CHCl_3 vapour treatments (Jenkinson, 1966b). Each treatment was followed by the addition of a small amount of soil infusion and an incubation to get rid of easily decomposable matter. Figure 3 illustrates the declining effect of seven successive CHCl_3 vapour treatments on CO_2 evolution from the soil as a consequence of a more and more reduced biomass (Jenkinson and Powlson, 1976). After the last CHCl_3 vapour treatment a more concentrated soil infusion was applied. Due to these treatments the original ^{14}C -labelled biomass was then replaced by another, which was not or only very weakly labelled.

After 1 day a solution containing 100 mg glucose was injected. Immediately thereafter the decomposition of the glucose began but no clear extra decomposition of labelled material was observed. No priming of the decomposition of dead organic matter after addition of glucose occurred.

So as to be more certain that living biomass and not humus had been involved in the "priming effect" in our experiments, we cultivated a labelled biomass in perlite. A mineral nutrient solution of 10 ml was added in the incubation vessel to 6 g pure perlite together with 41 mg glucose, $\text{D}-[^{14}\text{C}(\text{U})]$ - and 50 μl soil infusion. The CO_2 and $^{14}\text{CO}_2$ evolved during the incubation and the specific activity of the evolved CO_2 are given in Fig. 4. Two weeks after the start of the experiment 41 mg unlabelled glucose was injected. This resulted in a glucose- CO_2 peak as usual, but also in a small peak in the $^{14}\text{CO}_2$ curve (Fig. 4B).

In 2 weeks 75% of the added labelled glucose-C appeared as $^{14}\text{CO}_2$; 25% remained in the biomass and presumably in small amounts of metabolites. The small peak in the $^{14}\text{CO}_2$ curve is thought to be due to conversions in the living biomass. The "priming action" (Fig. 4B) began almost immediately after the addition of glucose and shows a typical peak. It suggests that the newly-introduced energy and building material leads to rapid biosynthesis in the already starving cells hereby also replacing some labelled material which is used for energy production and thus appears as $^{14}\text{CO}_2$. Of course the possibility cannot be excluded that consumption of dead cells and excreted metabolites play a role in the $^{14}\text{CO}_2$ peak. It can be assumed, however, that most of these substances were

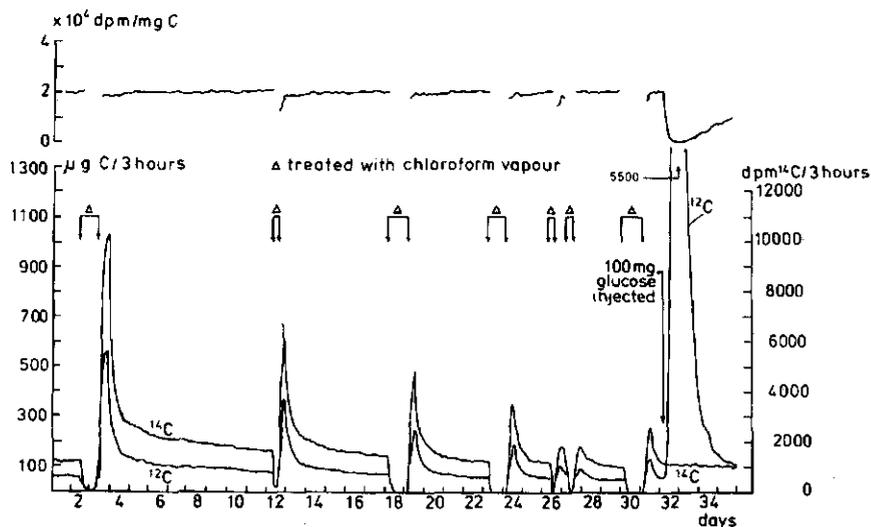


Fig. 3. The effects of successive treatments with CHCl_3 vapour on the decomposition of soil organic matter i.e. killed biomass. After the labelled biomass has been replaced by an unlabelled one (from soil infusions), no extra decomposition of labelled material occurs after addition of glucose.

already consumed by the starving biomass before the unlabelled glucose was given.

Another interesting feature is shown in this experiment, viz. the isotope fractionation in metabolism. Due to a higher atomic weight of ^{14}C , a discrimination against ^{14}C takes place, leading to a retardation of ^{14}C in metabolism.

The specific activity of the labelled glucose, on which a labelled biomass was grown in perlite, was 1.05×10^5 dis min^{-1} mg^{-1} C. The specific activity of the CO_2 evolved was 0.85×10^5 dis min^{-1} mg^{-1} C when consumption of the labelled glucose was highest. After that the specific activity gradually increased to 1.05×10^5 dis min^{-1} mg^{-1} C. In the beginning of this experiment the isotope effect amounted to 19%.

This means the CO_2 evolved at that time contains 19% less ^{14}C than the glucose from which it originated. The isotope fractionation in this case leads to a biomass and a residual organic matter with an enhanced ^{14}C content.

The isotope effect is presumably also responsible for the large difference in specific activity between the $^{14}\text{CO}_2$ evolved in the former experiment and the soil organic matter, viz., 2.0 and 2.7×10^4 dis min^{-1} mg^{-1} C, respectively.

DISCUSSION

From the results of our experiments it appears correct to assume that the addition of glucose to the

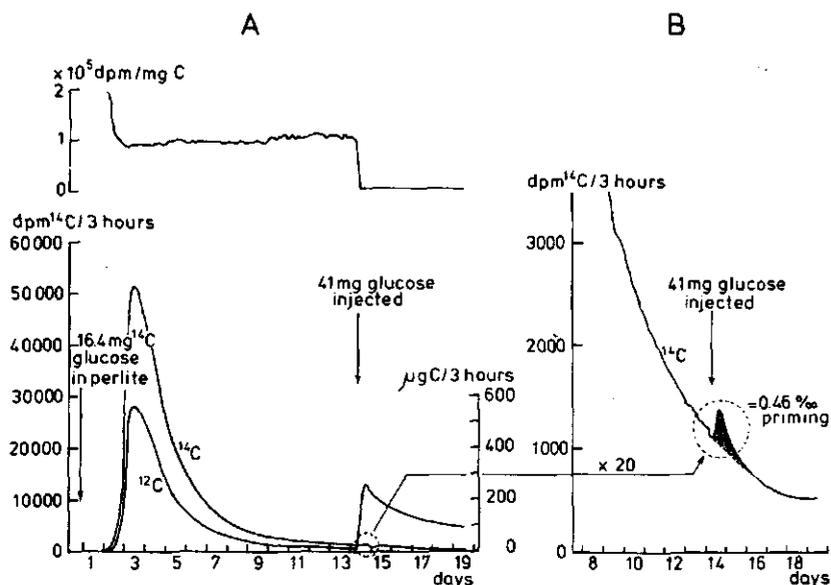


Fig. 4. (A) Growing a labelled biomass from ^{14}C -glucose in perlite. (B) The effect of addition of 41 mg glucose to the labelled biomass is given and represented in an enlarged form.

labelled soil led to a priming effect, which was due to conversions in the living biomass, whereby labelled material in the cells was respired at an enhanced rate during a short time. It is not yet clear why glucose additions have this effect.

The decomposition of dead biomass probably did not play an important role in our experiments. If this occurred, one would have expected a longer lasting effect, caused by the degradation of more difficultly decomposable parts. Figure 3 shows that after the biomass in the soil had been killed and the peak in the decomposition had passed, a gradual and slow decrease in the $^{14}\text{CO}_2$ production took place. This $^{14}\text{CO}_2$ production was higher than the basal respiration and was caused by the decomposition of less easily-degradable material. An effect like this did not appear after glucose addition.

Reports on comparable experiments in the literature are scarce. In most cases labelled glucose (Jansson, 1960; Shields *et al.*, 1974) or different fractions of labelled plant material (Szolnoki *et al.*, 1963) were added to soil a short time before the priming effect of a glucose addition was studied, Jansson (1960) found insignificant priming effects of glucose additions to two different soils with a freshly-labelled biomass. Szolnoki *et al.* (1963), using a percolation technique, found that a priming effect depended on the fraction of the labelled plant material decomposed in the soil during the 6 weeks before the effect of glucose addition was studied. To what extent the priming effect depended on the amount and type of stimulated biomass is not yet clear.

Shields *et al.* (1974), grew a ^{14}C -labelled biomass in a soil 2 months before the addition of ^{13}C labelled glucose and found a relatively slightly accelerated decomposition, lasting 3 days, of ^{14}C labelled material, defined as metabolites. A more important priming, however, took place in the unlabelled fraction, lasting from the second to the seventh day.

To what extent the biomass played a role as a (principal) source of C in the priming effect of the experiments cited is difficult to estimate. If the biomass were an important source of C in the priming effect, the extent of the effect would be more dependent on the amount of biomass that can be activated, than on the amount of added material, provided the addition would be sufficiently large to supply all members of the biomass that can be activated.

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